

The Cytoplasmic Inclusions of the Germ-Cells.

PART III. THE SPERMATOGENESIS OF SOME OTHER PULMONATES.¹

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With Plates 16-18 and 3 Text-figures.

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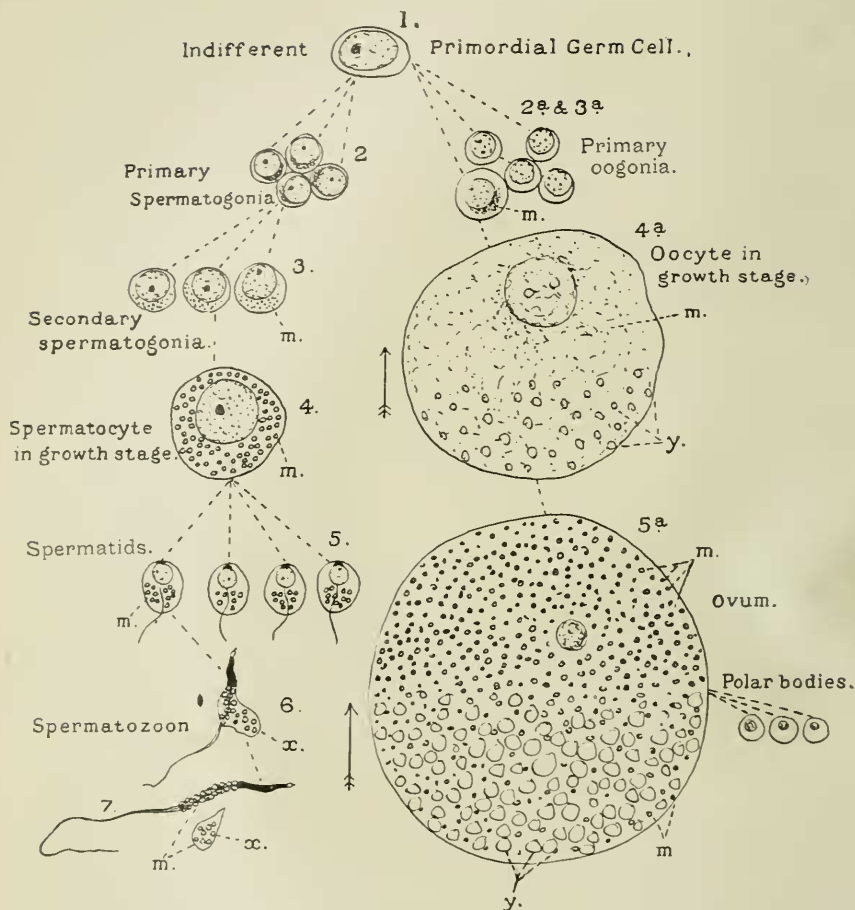
INTRODUCTORY.

IN the two previous papers of this series I have thoroughly examined the mitochondria and other cytoplasmic bodies in the gametogenesis of certain moths and butterflies, and of the garden snail. The aim of these papers, and of those which will follow, is to describe, to analyse, and if possible ascertain the functions of the various bodies in the cytoplasm. In my paper on the germ-cells of Lepidoptera, I described for the first time the remarkable acroblasts, bodies which give rise to the perforatorium, and I also gave the first correct account of the mitochondria during the metamorphosis of the spermatid into the spermatozoon. In the snail (*Helix aspersa*) I showed how and when the mitochondria first appear in the gametogenesis, and I further registered the discovery of two sorts of mitochondria which I found in the spermatid. This work has now gone far enough to enable us to give a cursory review of the mitochondria and several other bodies in gametogenesis—that is to say, a review of the behaviour of the plasmatic bodies during the time which the indifferent germ-cells are growing and differentiating to form eggs and spermatozoa. In *Helix* the indifferent germinal cells did not seem to contain any body which could safely be regarded as the rudiment of the future mitochondria; just as in most other cells in a resting condition, the nucleus had to one side of it a denser region of the cytoplasm which I identified as the archoplasm, or the usual dense cytoplasmic sphere which surrounds the centrosome of the cell. Apparently, though

I could not be sure, this region in which lay the archoplasm and centrosome, was where the first sign of the mitochondria appeared.

The mitochondria in these cases were not found to appear from the first as granules; the first sign of their coming was in the formation around one side of the external surface of the nuclear membrane of a dense cloud; this cloud was apparently at first more fibrous than granular, but little by little in it could be seen to appear grains of a definite nature. These were the mitochondria; they gradually became clearer, and soon the dense cloud which generally spread around the total periphery of the nucleus seemed to be absorbed, and the individual granular mitochondria were left (refer to Text-fig. 1). In spermatogonial divisions the mitochondria were seen to be fairly equally, though in a haphazard manner, divided between the daughter-cells. After the primary divisions such as all germ-cells undergo (multiplicatory phase), the mitochondria spread out throughout the cytoplasm and in the spermatogonial cell, remained granular and spherical (Text-fig. 1); in the next or growth stage of the germ-cell the spermatogonium or oogonium passes imperceptibly into what is called the spermatocyte or oocyte of the first order. In this growth stage the mitochondria were thought to increase in number, though because of their small size this could not be ascertained. In the spermatocyte, as the whole cell grew in size, so did the mitochondria become larger and more conspicuous; in some cases there was absolutely definite evidence for believing that each mitochondrial granule in the spermatocyte consisted of two parts—a cortical stainable area and a chromophobe medullary zone. In the oocyte the mitochondria from the first (just about or after contraction figure or synizesis, of the nucleus), became flocculent and finely granular, and of a different appearance from those of the spermatocyte (Text-fig. 1). In the latter the growth stage is followed immediately, as is well known, by two divisions. These two divisions, which give rise to four smaller cells, are called the maturation or first and second spermatocyte

TEXT-FIG. 1.



Diagrammatic scheme of gametogenesis with special reference to the Mitochondria. At 1 is an indifferent primordial germ-cell. After the sex of the embryo is determined, the primordial germ-cells become either primary oogonia or primary spermatogonia. At 2 are several primary spermatogonia derived from the original undifferentiated germ-cell. Each primary spermatogonium divides to give rise to several secondary spermatogonia (3). In some animals the earliest germ-cells contain some mitochondria (chick, frog?), in others (probably the majority) the earliest germ-cells may not contain differentiated mitochondria (*Helix*, *Insecta*?); in the latter cases the mitochondria appear in the cytoplasm as a cloud, at some

divisions. In one of them (second) the chromosomes are halved, in the other (first) whole chromosomes are bodily separated; this is the reducing division. In these two divisions the mitochondria lie in the cytoplasm around the mitotic figure or amphiaster, and each of the daughter-cells gets a certain number of the individual mitochondrial grains (Text-fig. 1). There is no evidence for supposing that each daughter-cell gets half of a mitochondrion, as is the case in ordinary mitosis with the chromosome. The spermatocyte divisions finished, there are left four cells each containing an approximately equal amount of the mitochondria; these four

stage between 1 and 3 in Text-fig. 1. Between 3 (secondary spermatogonium) and 4 (spermatocyte) is the growth stage, during which the mitochondria become larger and in all probability divide by fission, and so increase in number. The spermatocyte divides twice to give rise to four spermatids (5). Each spermatid has sorted out to it one-fourth of the granules of mitochondrial nature in the spermatocyte (5, 6, and 7). Now come the spermatoleosis stages (see page 209), which have no parallel in the development of the ovum. The mitochondria partly form the tail of the sperm (*m*.) and partly slough off (*x*).¹ At 7 is the ripe sperm. On the right are the oogenesis stages. There are not always the same grades in the multiplicatory phases in the oogonium (2 A and 3 A) as occur generally in the spermatogonial generations. Some of the oogonial cells in some animals appear to become specialised to nourish one of their fellows, which is then the young oocyte (4 A). The mitochondria disperse out, becoming flocculent or in the form of fine rodlets or strings. Yolk begins to appear, and in certain forms already examined (frog, etc.) both yolk disclets and mitochondria tend soon to become segregated in certain regions. The arrow points from the vegetative to the animal pole: the mitochondria in 4 A are as yet flocculent and fine. In some forms they remain so, in others they later become coarser and spherical (5 A), and though these granules are dispersed in both animal and vegetative poles, it is generally found that they tend to lie mostly in the upper or middle regions of the egg. The polar bodies are given off, and apparently never contain any important part of the mitochondrial grains (Rana). At 5 A the ripe egg is drawn, the yolk (*y*) and mitochondria (*m*) being shown roughly as they are found, but these stages have never been studied thoroughly enough for one to give a diagram which would apply generally. The drawing in 5 A, however, serves to show that the mitochondria are not evenly dispersed throughout the egg plasma.

¹ It should, however, be pointed out that in some forms, possibly all Insecta and Prosobranchia for instance, there is no sloughing off of any mitochondrial matter. This may apply also to Mammalia and Scorpions. The case of Mammals (*Mus* and *Cavia*) is doubtful.

cells or spermatids now begin the last stage of the history of the formation of the ripe spermatozoon; the nucleus shrinks and undergoes certain staining changes, while from the centrosome grows the axial filament or tail of the spermatozoon. The mitochondria in all animals so far studied (with the sole exception of *Peripatus capensis* (15), now group themselves in some way or other in connection with the head centrosome and the axial filament, so as to form a definite part of the tail of the sperm in the region behind the nucleus. I have been able to show that in the case of Pulmonate Mollusca part of the mitochondria of the spermatid is always sloughed off with the rejected cytoplasm; this is a fact of the very first importance. (See description to Text-fig. 1.)

In the case of the egg, during growth the flocculent mitochondria gradually disperse through the furthestmost parts of the cytoplasm, and they may later become more granular and larger. They do not metamorphose into yolk disclets, nor do they disappear; in some forms as *Ascidia*, the mitochondria during segmentation are segregated in special regions of the egg. In the maturation division of the egg the mitochondria take no very definite part as far as is known. In some unpublished work on the maturation of the egg in *Rana temporaria* I found that the minute polar body cytoplasm did not contain any mitochondria, but only the chromosomes of the maturation. No mitochondria are rejected in this way, as chromatin is rejected. This also is a fact of some importance. (For Oogenesis, refer to right side of Text-fig. 1.)

In both oogenesis and spermatogenesis there are in all animals certain other cytoplasmic bodies¹ which generally differ from both chromatin and mitochondria in their staining and fixing affinities. Whether such bodies in different orders and families of animals are homologous and identical one cannot at present say. In spermatogenesis such bodies may ultimately form part of the ripe sperm, but their function is at present obscure.

It will now be seen that in the genesis of egg and of sperm

¹ These are dealt with in a forthcoming paper.

the mitochondria are a constant and regular factor; they are undoubtedly of prime importance and there is no doubt that the correct solution of their function would be one of the most valuable of modern contributions for zoological and histological literature.

I regard the study of the mitochondria as being of great importance to the embryologist. Not only is their behaviour in gametogenesis worthy of notice, but in the methods which I have described here and in my previous papers, and which I have tried myself to improve, the embryologist has an instrument which will enable him to ascertain many remarkable facts relating to the division of the cytoplasm in the segmentation of the egg and in early organogeny.

In the case of delicate marine eggs especially should alcohol and acetic acid be rigidly avoided. Observers who study marine animals should carry out experiments with osmic acid, chromic acid, bichromate of potash, and formol mixtures. In some cases, two or three of these substances might be used successively.

TERMINOLOGY.

It appears that there is still great confusion in the terminology used by various writers, and unwittingly I myself may have added to the disorder. The main factors in producing such confusion seem to be that, with the advent of improved technical methods our conceptions as to the origin and homologies of numerous bodies in different forms have had to undergo drastic alteration, and that older terms have been used several times by various authors in differing shades of meaning. It is merely in order to clear away some of these cobwebs that I give the following explanations and propose the following terms. Apart from the question of the mitochondria, one example will serve to show how great a confusion exists in many branches of cytology: The "centrosome" (centriole?) or central spot in the asters of a mitotic figure), when in the "resting" cell has around it a cloud of stainable matter. This apparatus (or hypothetical bodies

conceived in connection with it) has been called Archoplasm, Archiplasm, Centropiasm, Attraction Sphere, Centrosphere, Astrosphere, Idiozome, Kinoplasm (Macrocentrosome), Periplast, etc. (see Wilson's "Cell," p. 323, etc.).

This is most complicated, and the various shades of meaning conceived by the inventors of these words are very difficult to understand. Unfortunately, however, this is not all; in the same connection there are the confused questions as to the relationship of "archoplasm" or "idiozome" to the acrosome or head of the sperm. Moreover, some of these words have others so like them, used in a totally different sense, that needless confusion is introduced, e. g., idiosome and idiozome.

In this short glossary no attempt is made to give exhaustive and useless references.

Apart from the fact that numerous terms have been coined for bodies of identical nature in different animals, there are certain terms which have been invented by observers of the Protista. For instance, the word centroplast refers in the Heliozoa to a body which Schaudin showed in *Acanthocystis* to act in mitosis exactly as the Metazoon centrosome. Schaudin's account has lately been confirmed by Dobell ('Quart. Journ. Micr. Sci.,' vol. 62, part iv), who, curiously enough, instead of seeing in this added support to Schaudin's theory on the phylogeny of the centrosome, is sceptical with regard to the view that the centroplast of Heliozoa is homologous with the centrosome of Metazoa.

GLOSSARY.

Apyrene, Eupyrene, Oligopyrene Spermatozoa, and Typical and Atypical Spermatozoa.—Eupyrene and Typical spermatozoa are ordinary normal sperms.

Atypical spermatozoa may be either "Apyrene" when the nucleus for some reason has altogether degenerated and is absent, or "Oligopyrene" when by some process only part of the chromosomes go to form the sperm nucleus ('Quart. Journ. Micr. Sci.,' vol. 62, part iii, p. 465). Some authors are in favour of discarding Meves' terms Oligopyrene, Apy-

rene, and Eupyrene, in favour of the simpler terms Typical and Atypical spermatozoa. I am in agreement with this, especially in view of the fact that I deny that such spermatozoa have the significance attached to them by Meves and others (Gatenby, 'Quart. Journ. Micr. Sci.,' vol. 62, p. 481).

Archoplasm, or Archoplasmic Zone.—The dense mass of protoplasmic material gathered around the centrosome in the form of a sphere. Meves' word "idiozome" is applied to the large archoplasmic zone of the spermatid centrosome, and as there is no doubt that the archoplasm of both somatic and germ-cells is identical, it is suggested that Meves' term "idiozome" be discarded in favour of the word "archoplasm," or archoplasmic zone. Other words which have been used in almost if not quite identical sense as Archoplasm are "attraction sphere" and "astrosphere," or simply "sphere"; the former is quite commonly used in connection with germ-cells. Not only is Meves' term superfluous, but it is too much like the word "idiosome," which means the same as pangen or biophore.

Acrosome and Acroblast.—The perforatorium or acrosome is the small body or cap at the extreme tip of the ripe sperm. This body has been traced back by me to the young spermatocyte ('Quart. Journ. Micr. Sci.,' vol. 62, p. 438) and is in all earlier stages separate from the nucleus. It is only later that it becomes fused to the head of the metamorphosing sperm nucleus. It is proposed to call any body found in the cytoplasm of the male germ-cell which finally gives rise to the acrosome or perforatorium—an acroblast ('Quart. Journ. Micr. Sci.,' vol. 62, p. 418, footnote), and it is not till the acroblast or acroblasts have fused to form the cap of the spermatid nucleus that they (or it) form the acrosome. On Pl. 25, fig. 41, of 'Quart. Journ. Micr. Sci.,' vol. 62, I should call the bodies *A.B.* acroblasts, the fused body in Pl. 25, fig. 42, *A.C.*, an acrosome.

Cytoplasmic Bodies, or Plasmatic Bodies.—Terms apply to all cell elements other than the nucleus (Chromatin). This would include centrosome, archoplasm, mitochondria,

and the various other bodies of an enigmatic nature found in the cytoplasm of germ-cells. I do not think that yolk discs or fat globules come under this term; I would call the latter "dentoplasmic bodies," for they are obviously not such definite structures as the mitochondria or chondrioplasts.

Chondrioplast.—The archoplasm in the germ-cells of some animals is often studded over its periphery with a number of banana-shaped rodlets or batonnettes. The whole apparatus (archoplasm included) has been called the "nebenkern" (see "Nebenkern" in this glossary). The term "chondrioplast" is singular number and refers to one of the rods or batonnettes. The adjective would also be chondrioplast, the entire rods together would form the chondrioplasm. The rods would be spoken of as chondrioplast batonnettes or rods, or simply chondrioplasts. The name also draws attention to the fact that these bodies are akin to the mitochondria in fixing and staining affinities. What was originally called the "nebenkern" of pulmonates would under this nomenclature be spoken of as "chondrioplasts and archoplasm."

Chondriosome, Chondriokont, and Mitochondrion.¹—The bodies signified by these names² have been found in the cytoplasm of the germ-, nerve-, gland-, blood-, muscle-, and connective-cells, etc., of all animals. They are in the forms of rods or granules, are dissolved by acetic acid, alcohol, chloroform, and ether, resist nitric acid (circa, 3 per cent.), are sometimes turned brown by osmic acid (rarely black), are preserved by the chromium salts, osmic acid, and formalin, and are stained *intra vitam* by janus green, neutral red, etc. The mitochondria in fresh unstained material look like slightly refractive rods or granules and can often be noticed in flowing movement.

¹ Regaud and Fauré-Frémiet both consider the mitochondrion chemically a combination of phospholipin and proteid.

² Mitochondria (*μίτρος*, a thread; *χόνδρος*, a grain) are granular; chondriokonts (*κοντός*, a pole) are rod-like. Chondriosome (*σῶμα*, a body) is a general term applied to both varieties. The word mitochondrion is now used for a body of any shape and is more frequently used than chondriosome.

It must particularly be noted that there are other cytoplasmic bodies such as chondrioplasts ("nebenkern" batonettes of Pulmonates), acroblasts, and certain siderophile granules, which are not exactly mitochondrial, but which are very nearly of the same chemical nature. All these bodies and the mitochondria act in almost the same way under treatment of acids and fat solvents.

The mitochondria can be distinguished from these bodies only by watching their behaviour, or by the fact that such pseudo-mitochondrial elements are of a different size or shape from the mitochondria. In certain cases mitochondria cannot histochemically be distinguished from the zymogen granules of giant cells.

Nebenkern.—This term has been applied to at least three different cell elements.

(1) The mitochondrial body in the spermatid of Insecta ("Macromitosome," 'Quart. Journ. Micr. Sci.,' vol. 62).

(2) The chondrioplasts and the archoplasm of Pulmonate and other Molluscs. (These are not the mitochondria.)

(3) The secondary nuclei in the egg of an ant, *Camponotus* (Blochmann): such secondary nuclei are unrelated either to chondrioplasts or mitochondria.

(4) The micronucleus of some Ciliates has been called "Nebenkern" by Butschli (1876) and by Hertwig later.

(5) The enigmatic secondary "nuclear" body in *Paramœba eilhardi* has been called "Nebenkern" by Schandinn.

(6) The term has also been applied to cytoplasmic structures in gland and nerve cells.

It is clear that the greatest confusion exists with regard to the word "Nebenkern"; in fact, some authors have applied this term in an extremely slipshod manner to any enigmatic body lying at any time near the nucleus. A convenient rendering of the term "Nebenkern" would be "paranucleus." The body in insect and other spermatids called "Nebenkern" was at first thought to be derived from spindle-fibres; it is really mitochondrial, and I have already proposed the term "macromitosome" for it (see "Macromitosome").

The word "Nebenkern" has been used so loosely that the application of it to any structure occurring in gametogenesis only produces confusion, and it is recommended that cytologists discard it in favour of other well-defined terms.

Nest.—The primary spermatogonium (see "Spermatogonium") gives rise by a number of divisions to a group of cells (secondary spermatogonia), which generally all develop side by side in the form of a "nest" or definitely isolated group of cells. (For a spermatogonial nest see Gateby, 'Quart. Journ. Micr. Sci.,' vol. 62, Pl. 25, fig. 46.)

Middle Piece.—This term is used in connection with that region of the sperm just behind the head centrosome. In the spermatozoon of mammals, the nucleus is generally a spatulate shape; behind it comes a short region which lies between the head and second centrosome. This part is apparently the mitochondrial region of the sperm-tail, and is, strictly speaking, the middle piece; behind this region is the rest of the sperm-tail. In Molluscs and certain other animals there is no such arrangement; in these cases the entire tail has formed around it a mitochondrial sheath, and thus the entire tail in the mollusc is the homologue (as far as mitochondria are concerned) of the short region behind the head centrosome of the mammalian sperm. In all sperms there is a region behind the nucleus where the head centrosome lies—the second or tail centrosome in some molluscs and insects seems to become sloughed off or absorbed—but it should be remembered that there would be difficulty in identifying a special middle piece such as in mammals, throughout the animal kingdom. This is particularly the case with Invertebrates.

Macromitosome.—In many Arthropods, and apparently in all the Insecta, the mitochondrial bodies of the spermatid become grouped to form either a remarkable spireme or a definite figure of some kind, or macromitosome. This body has been called a "Nebenkern," but for reasons given above, under the heading of "Nebenkern," it has been thought

better to avoid the further use of this confusing term. (For a macromitosome, see 'Quart. Jour. Micr. Sci.', vol. 62, Pl. 23, fig. 17, *M.D.*)

Spermatogonium, Spermatocyte, Spermatid, Spermatozoon.—For all these terms, see Wilson's Text-book on 'The Cell.' In using the words primary spermatogonium and secondary spermatogonium, one refers to the fact that in many animals the primordial or indifferent germ-cells in the future testis generally can be distinguished as undergoing a definite number of division grades. Thus the embryonic or primordial testis, which may often be distinguished from the primordial ovary by its shape or by the manner in which its cells are grouped, contains a certain number of cells—or primary spermatogonia; generally these are not as yet grouped in cysts or nests (see 'Nest'). Later, they undergo further divisions, and become grouped in cysts; in all probability one primary spermatogonium gives rise by itself to a group of secondary cells or secondary spermatogonia, which become grouped to form a definite cyst of cells. The secondary spermatogonia generally pass on to the growth stage, becoming spermatocytes (see Hegner, 'Germ-cell Cycle in Animals,' Macmillan, 1914).

Spermatogenesis.—This term applies in all modern literature to all the changes which lead to the formation of a ripe sperm from a primordial germ-cell. There is a specially important period in spermatogenesis known as the "metamorphosis of the spermatid into the spermatozoon." This period is especially important, since up to the spermatid stage the sperm-cell is like any other cell in appearance; at this period there begin those remarkable changes which end in the formation of the spermatozoon. As there is no special term for the expression "metamorphosis of spermatid into spermatozoon," I propose the term "spermateleosis"¹ to mean the changes during which the ordinary spermatid cell becomes converted into the peculiar flagellate organism known as the spermatozoon. This term is really needed in cyto-

¹ From *σπέρμα*, and *τέλειω*, I finish.

logical literature; many of the papers on spermatogenesis deal especially with this stage, during which the nucleus and cytoplasmic bodies become so profoundly altered.

Spireme: Macromitosomal or mitochondrial spireme.—The word spireme means something coiled, or a skein. Generally applied to the nucleus at a time when the chromatin forms a coil. I have used it in connection with the mitochondrial coil (macromitosome) of Lepidoptera.

Spindle-bridge.—The spindle fibres in the telophase of division often condense to form a body joining the two daughter-cells long after the division has taken place. This body is the spindle-bridge. Equivalent to the German "Spindelrestkörper" and the French "reste fusorial." For a spindle-bridge, see Gatenby, 'Quart. Journ. Micr. Sci.,' vol. 62, Pl. 25, fig. 46, S.B.

For a number of other terms widely used in cytological literature, one may consult the excellent "Glossary" in Wilson's text-book on 'The Cell,' where an extensive bibliography is given.

MATERIAL, METHODS USED, AND SPECIES STUDIED.

The technique used by me hardly differs from that already described in the previous paper (1). Where any differences exist, I have indicated in the section of the paper immediately concerned.

The Pulmonates studied were as follows: *Helix aspersa*, *H. nemoralis*, *H. rufescens*, *Limax maximus*, *L. agrestis*, *Arion ater*, and *A. hortensis*. I also sectioned and studied less thoroughly *Limax marginatus*. It will be noticed that I have chosen in each genus a large species and a small one. This does not apply to *Testacella*, where I could only get one species.

The length in millimetres of the species examined by me is as follows, the average being given:

(<i>Limax maximus</i>	125
(<i>Limax agrestis</i>	55

{	Arion ater	57
{	Arion hortensis	20
{	Helix aspersa	60
{	Helix nemoralis	40
{	Helix rufescens	17
	Testacella haliotoïdes	65

In different localities the average sizes of the slugs (*Limax* and *Arion*) vary considerably: the figures given for *L. maximus* represent the average size of about six specimens used by me; very often *L. maximus* attains a size of 6 in. (153 mm.).

Following the classification given by Pelseneer in 'Lankester's Treatise on Zoology,' part v, Mollusca (2), we find that the Families, *Helicidæ*, *Limacidæ*, and *Arionidæ*, all belong to the Tribe *Holognatha*. In the latter the jaw is simple, without a superior appendage. *Testacella* is grouped in the possibly polyphyletic Tribe, *Agnatha*; in this there are no jaws, the radular teeth are narrow and pointed.

In the *Limacidæ* and *Arionidæ* differences in external morphology are not at first very obvious. Both *Limax* and *Arion* are "slug-like." In *Arion* the pulmonary opening is towards the front end of the mantle, while in *Limax* it is nearer the hind end; in *Arion* there is a tail mucous gland, which is absent in *Limax*. The *Limacids* have a definite shell covered by the mantle (shield), but in the *Arionidæ* the shell is amorphous, being formed of granules. Though the differences between the *Limacidæ* and the *Arionidæ* are not great, they are distinctive when once grasped, and it is impossible to confuse the two genera. Nevertheless, it must be said that the two genera are undoubtedly very closely allied. *Testacella*, in outward appearance, is quite distinct; the *Helicids* are also quite a well-marked type. How far the outer morphological differences are correlated with the differences in the germ-cells and mitochondria will be shown below, and I will endeavour to analyse how far important are the size differences with relation to the mitochondria in the three genera in which I have been able to study giant and small forms.

The Arionidæ (the Post-nuclear Granules).

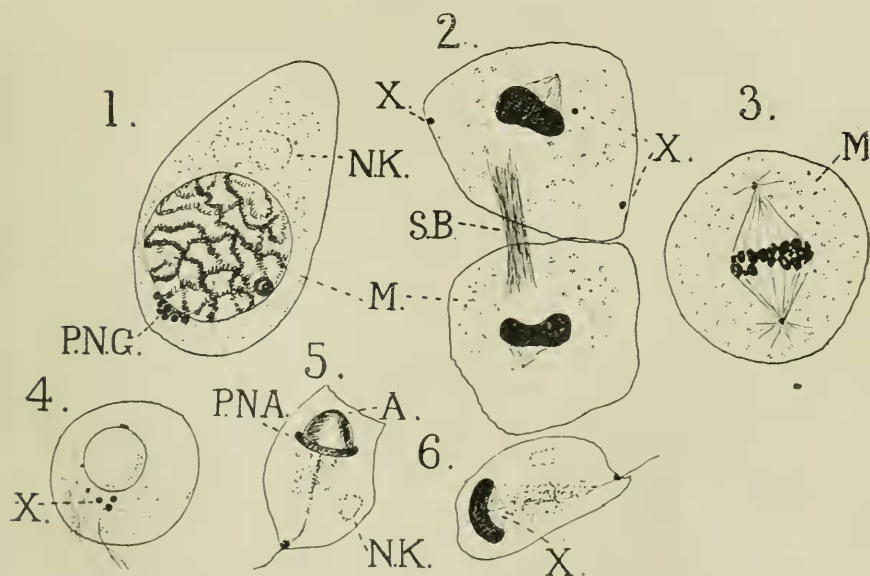
The case of the Arionidæ will be treated first because it is in this Family that certain new bodies found by me are best developed.

If the metamorphosing spermatid of *Arion ater* or *A. hortensis* be examined at such a stage as that in Pl. 16, figs. 15 or 16, it will be noticed that at the back of the nucleus lies a darkly staining, coarsely granular body marked *P.N.G.* From their position I have called these structures the post-nuclear granules. When once the appearance of the post-nuclear granules has been learnt, it is an easy matter to find them in the spermatocyte, especially in sections which have been differentiated till the mitochondria are greyish. The granules may then be easily discovered, since they retain the stain more than the mitochondria and, consequently, appear black (Pl. 16, fig. 15, *P.N.G.*). The position in which I have drawn these granules, both in Pl. 16, fig. 11, and in Pl. 16, fig. 12, is quite characteristic. In a bundle of spermatocytes attached to a part of the germinal epithelium it will almost invariably be found that the chondrioplasts lie towards the epithelium, while the post-nuclear granules are towards the lumen of the gland. If the spermatocytes are examined just at or after the prophases of the heterotypic division, the post-nuclear granules (hereafter called P.N. granules) are very clear, as in Pl. 16, fig. 11).

Now, if the Arion's ovotestis be preserved for eighteen to twenty hours in my modification of Flemming, then cut $6\ \mu$ in thickness and stained with eosin and toluidin blue, the mitochondria are reddish, the chromatin is blue, and the P.N. granules blue. The slide is first flooded with a .5 per cent. solution of eosin, and warmed till steaming for about half a minute. The superfluous eosin is drained off and a .75 per cent. solution of toluidin blue added. This is heated in the same way for about one minute; afterwards the toluidin blue is poured away, the slide drained and blotted. It is then quickly differentiated in 90 per cent. alcohol, dipped in absolute,

then into xylol, and mounted. The P.N. granules stain just as densely as the chromosomes in the metaphase, and can be picked out with a one-sixth objective quite easily. The mitochondria are stained by the eosin a red colour, but are not sharply differentiated. The chondrioplast also is vaguely reddish in colour like the mitochondria. By this means it has been possible to follow out the history of the P.N. granules.

TEXT-FIG. 2.



The ovotestis was fixed in Flemming fluid without acetic acid, and stained in eosin followed by toluidin blue. The mitochondria stain vaguely reddish, the post-nuclear granules (*P.N.G.*) of the spermatocyte are blue (Fig. 1). In the prophases of the maturation division and during division (Fig. 2) the post-nuclear granules cannot be found. In rare cases one can find granules (*X.*), which I do not think are the post-nuclear grains. In Fig. 3 a second maturation division metaphase is drawn showing mitochondria vaguely represented, and absence of post-nuclear grains. In Fig. 4 is a young spermatid in which the *P.N.Gs.* are grouped behind the nucleus (*X.*). In Fig. 5 the *P.N.Gs.* have fused to form a collar behind the nucleus; the post-nuclear matter stains less densely, now a purplish colour, and by the stage in Fig. 6 the zone behind the nucleus marked (*X.*) appears to stain reddish, no blue colour being perceptible. *N.K.* Chondrioplast. *S.B.* Spindle-bridge.

These first make their appearance a little before the pro-phases of the heterotypic division begin. They reach their maximum size and density between the stages drawn in Pl. 16, figs. 11 and 12, when they form a more or less grape-like mass, disposed as already explained. When the spermatocyte enters the pro-phases of the first division the P.N. granules are nowhere to be found, and they certainly disappear before the chondrioplast batonnettes become disturbed prior to nuclear division.

Afterwards there is to be found in the young spermatid a number of granules which are almost certainly of the same nature as those in the spermatocyte, though it is impossible to follow them through the maturation divisions to make quite sure.

In *Arion hortensis* I could count eight granules in the spermatocyte at the pachytene stage.

After their reappearance in the young spermatid, the P.N. granules are generally found grouped, as shown in Pl. 16, fig. 13, in *A. hortensis*. At this stage they form an irregularly spherical mass of granules, the same size as the mitochondria, but staining more heavily. In the next stage the P.N. granules move towards the nucleus and become grouped to form a plate, as shown in Pl. 16, fig. 14. They are then spread out like a layer of marbles, whose individual elements are fairly regularly, but not quite evenly, disposed. The centrosome divides at about this time, but I have not specially studied the events connected with the centrosome at this period. A little later the P.N. granules close up together and form a solid plate, whose centre is pierced by the first centrosome and the axial filament (Pl. 16, fig. 15). The granules have hitherto been separated from the nucleus by a small space. In the next stage the post-nuclear apparatus becomes applied to the posterior surface of the nucleus, as shown in Pl. 16, fig. 16. The P.N. apparatus is now at its most conspicuous stage and cannot be overlooked. In Pl. 16, figs. 15 and 16, it will be seen that apparently the substance forming the P.N. apparatus is much larger in extent than that

in the early spermatid, as for instance, in Pl. 16, figs. 13 and 14. Very often it is difficult to ascertain whether there is a dividing line between the mitochondria behind and the post-nuclear grains. In such a case as that in Pl. 16, fig. 16, often it looked as if the thickened P.N. disc had grown at the expense of some mitochondrial granules. Very soon after the formation of the solid disc behind the nucleus, the mitochondria become grouped to form a mass at the rear of the P.N. apparatus.

Gradually the spermatid nucleus becomes basophil, and the P.N. apparatus and acrosome become for a time obscured. When the nucleus again stains oxyphil the post-nuclear apparatus is found to have shrunk considerably, and it now forms a very small perforated disc just behind the first centrosome (see Pl. 17, fig. 27, *P.N.A.*).

There is no reason for doubting that the P.N. granules give rise to an apparatus which takes part in the formation of the ripe sperm, and their position in the latter is just behind the first centrosome.

In the figures given in Text-fig. 2 it might be thought that what I have described as P.N. granules are really a form of multiple centrosome, and the disappearance of the P.N. granules at the prophase of division might be explained by supposing that these structures form the centrosomes of the asters. Against the view that the P.N. granules are centrosomes there are three irrefutable facts.

(1) The centrosome is always near or inside the archoplasm, and centrosomic activity always arises within the archoplasm (see especially Bolles Lee's work, and my Pl. 16, fig. 5).

(2) The centrosome of the dividing pulmonate germ-cell is never multiple.

(3) The centrosome, in later stages of the spermatid, can be seen to be quite separate from the P.N. granules (for instance, Pl. 16, fig. 16).

It is clear, therefore, that the P.N. granules are not of a centrosomic nature.

A Comparison of the Mitochondria of Eight Species of Pulmonates.

In the first place it should be remarked that the Pulmonate Mollusc is not the best form for instituting a comparison in size of the mitochondria, principally because, in different parts of the ovotestis, the mitochondria tend to differ often quite considerably in size. This is because of the varying conditions of nutrition and the varying number of cell divisions which the generations of cells undergo. But, by examining a large number of cases, it becomes possible to gain a good idea of the average size of the chondriosome elements.

In my previous paper (1) I made a special study of the size differences in *Helix*, and my figures there are sufficiently explanatory. I have also found differences in *Limax* and in *Helix rufescens*. In all my preparations, here and there I found cells in which the mitochondria were undoubtedly in the form of hollow spheres (Pl. 16, fig. 4, and Pl. 17, fig. 24), and there is little doubt that the mitochondrial element of the Pulmonates consists of two parts—an inner, less colourable core, and a cortical layer of chromophil matter.

Comparison of the mitochondrial bodies of all the species treated here shows that in a given order, such as the Pulmonata, there is a general agreement in mitochondrial size, though the sizes of the animal forms may differ markedly.

The manner in which the mitochondria become grouped in the spermatocyte divisions, the grouping of these bodies in spermateleosis (p. 209), and their final disposition is throughout similar in every case. The same applies to their behaviour with regard to fixatives and to stains.

Though there is a tendency throughout for the sperm-tail to be spirally twisted, this tendency is more pronounced in *Testacella* than in *Limax*. In the slug-like forms there is a constant tendency for the mitochondria to become divided into two batches, one of which retains its position near the head of the sperm, while the other drifts down to the tail (see Pl. 17, fig. 22). This occurs strikingly in *Arion hor-*

tensis, apparently less in *A. agrestis*, and never in the Limacids. Such differences as can be demonstrated are, however, quite small. In Limacids, for instance, the mitochondria very characteristically become grouped behind the post-nuclear disc, as shown in Pl. 16, figs. 7, 8, and 10, while in other forms this grouping, though quite evident, seldom occurs so strongly as to produce perfectly constant and compact figures. In every form so far observed there is a residuum cast away at the end of spermateleosis. This is shown in Pl. 16, fig. 9, at *R.*; in Pl. 16, fig. 3, this residual globule is drawn to a much higher scale (see Pl. 17, fig. 26). The mitochondria in such globules generally stain lightly, and rarely quite so sharply, and appear to have become altered. In *Helix aspersa* I found that they became larger as they were gradually sloughed down the tail. Curious as it may seem, in *Limax agrestis* especially, I am inclined to believe that the mitochondria sloughed off in the residual globule are as numerous as those to be found in the spermatid. I am convinced that the mitochondria of Pulmonates do not bodily form the tail sheath as seems to be the case in Mammalia.

With regard to the important question of the formation of the tail of the sperm, in relation to the part played by the mitochondria, I carefully studied the case in *Testacella haliotoides*.

With quite low powers it is evident that the sperm-tail of *Testacella* is spirally twisted. In Pl. 17, fig. 25, the head and upper region of the nearly ripe sperm is depicted. If favourable examples be chosen it will be found that there is an inner rod (*C.R.*) surrounded by a spirally twisted sheath (*S.P.*). The spiral goes in the opposite direction to the hands of a clock; I found no exceptions to this.

I have noticed that it is a most difficult matter to ascertain the exact origin and relationship of these elements in the tail. In Pl. 17, fig. 30, is drawn a sperm at a stage when the spiral sheath (*S.P.*) is partially formed. Between the letters *X.-X.* and *Y.-Y.* is a region where the spiral gradually disappears;

now, inspection of the region *Z* shows that there is already formed around the axial filament a thicker rod; the latter seems almost certainly identical with the central rod drawn in Pl. 17, fig. 25, at *C.R.*

Careful examination of the region *X.* and *Y.* in Pl. 17, fig. 30, shows that the spirally twisted part of the sperm appears to be an outer purely cytoplasmic sheath, while the inner core (*Z.*) is mitochondrial in nature; nevertheless, I am unable to speak with certainty. In Pl. 17, figs. 31 *A, B, C,* and *D,* I have drawn various regions of the tail at different stages; Pl. 17, fig. 31 *A,* is typical of a lower region of Pl. 17, fig. 31, and Pl. 17, fig. 31 *B* is the kind of loose spiral, apparently formed of two strands, neither of which stained more heavily than its fellow.

Pl. 17, fig. 31 *C,* is a mid-region in a fully-formed sperm. Pl. 17, fig. 31 *D,* is another upper region in an early sperm. Examination shows that at first the spiral is loosely twisted, but later on it becomes telescoped up, and then appears as in Pl. 17, fig. 31 *C.* At such a stage it is impossible to form an idea as to the elements forming the spiral. Pl. 17, figs. 31 *A* and *D* appear to show that there is an inner dark rod around which the spiral part is coiled. In sperms at the stage drawn in Pl. 17, fig. 30, the spiral appears to fade into the region just where the cytoplasm is being "skimmed off" the rod; it should be pointed out that there are mitochondrial granules in this region as well.

The later sperm is drawn in Pl. 17, fig. 26, the beads of cytoplasm containing the plasmatic elements are eventually sloughed off. These beads contain granules which were the mitochondria of the spermatid, but they now often appear changed; the changes are sometimes ones of size, at other times ones of staining power. The chondrioplast does not seem to take direct part in the formation of the sperm-tail, though it is impossible to form a quite definite opinion. The behaviour of the mitochondria at the time when the post-nuclear apparatus is formed is of some importance. It has been noted in every case that the chondrioplasts take up a

position intermediate between the head and tail of the lengthening sperm; it is also evident that in such a case as *Arion hortensis* the mitochondria become divided into two groups. In *Helix aspersa* it has already been shown that the smaller mitochondria lie nearest the head centrosome in the lengthening sperm. All these facts mean that at the time when the sperm is lengthening some body at the posterior end of the ovoid nucleus is exerting influence on the mitochondria and chondrioplasts. The latter take up the position indicated in Pl. 17, fig. 22, probably because this is the region where the forces causing the lengthening tendency, and the force being exerted from behind the nucleus on the plasmatic bodies, are balanced. In the same way the smaller size of the micromitochondria enables them to be influenced more easily than the larger mitochondria, and hence one finds the grouping indicated in the figures in this and my previous paper. The probable reason why the mitochondria in *Arion* become split into two groups is that the forces being exerted from the region of the nucleus only succeed in attracting part of the mitochondria while the outgrowing tail end of the cell carries away the rest (Pl. 17, fig. 22).

In some cases, just at the prophases of the spermatocyte mitosis, the mitochondria seem to be definitely gathered around the archoplasm; the attracting body is probably the centrosome, which is breaking into activity.

In all Pulmonates examined by me it has been found that there exists a remarkable period during which the staining of the tail alters very greatly. If such a portion of the ovotestis wall as that drawn in Pl. 16, fig. 9, be examined, it will be found that the spermatids at different periods in spermateleosis do not agree in staining affinities. In Pl. 16, fig. 9, at 1 are spermatids in the stage drawn in Pl. 16, figs. 7 and 8; at 2 are spermatids drawn at a stage earlier than that in Pl. 16, fig. 6; and at 3 are spermatozoa nearly ripe. It is quite evident that the latter stain with greater intensity than any other stages. This increased affinity for stains is due to the tail sheath having been formed; it is

just at the time of formation of the sheath that this increased affinity is noticeable.

A Comparison of the Chondrioplast¹ Elements of the Eight Species of Pulmonates.

I have found that each species possesses the well-known chondrioplasts in the form of siderophil banana-shaped batonnettes. The number of rods can be fairly easily counted in the spermatids of some species.

The numbers seem as follows :

Limax agrestis, 2 batonnettes in the spermatid.

Limax maximus, 2 ; rarely 3.

Arion ater, 4 to 8.

Arion hortensis, 4 to 8.

Helix aspersa, 6 to 8.

H. nemoralis, 6 to 8.

H. rufescens, from 5 to 7.

Testacella haliotoïdes, from 6 to 14.

From the case of the Limacidae especially it is evident that in the spermatocyte divisions the number of batonnettes is halved at each mitosis. The *Limax* spermatid has two rods, the spermatocyte eight (Pl. 16, figs. 1 and 7 or 10). There is a greater difference between the chondrioplast of *L. agrestis* and *L. maximus* than between these bodies in the two Arionidae. In *L. maximus* the batonnette is slim, while in *L. agrestis* it is much stouter and more curved. In the Helicids and *Testacella* the rodlets are often approximately of the same type. In Arionids the chondrioplast rods form a fairly characteristic body, being stumpy ; their ends appear to fuse and cause the body of individual batonnettes to be less well defined than in other forms (see Pl. 16, figs. 15 and 17). In *Testacella* the rodlets, though very like certain Helicid types, are yet finer and more numerous. In fact, it can safely be said that each family has a distinct type, and even specific differences may be found (e. g. Limacids).

¹ Chondrioplasts and acroblasts will be discussed in the light of "Golgi-Kopsch Apparatus" in a future part of this series.

Though it cannot be quite definitely stated, since there is no accurate method of judging, in all probability the amount of chondrioplast material is about the same in the eight species; that is, of course, proportionately to the size of their cell.

In every case the chondrioplasts take up their position half-way between nucleus and tail centrosome in the stages drawn in Pl. 16, figs. 7 and 8, 16 and 17, and Pl. 17, figs. 19, 21 and 22.

This has already been commented upon; in certain cases (*Arion hortensis*) it seems as if the chondrioplasts were partly responsible for the sorting out of the mitochondria into two groups. In Pl. 17, fig. 18, the chondrioplasts at X. mark the position where the division into two groups takes place. In Pl. 17, fig. 20, the chondrioplasts are not at this place, but in Pl. 17, fig. 22, they are all in the characteristic intermediate position.

With regard to the very remarkable differences in the shape and size of the individual chondrioplasts in *Helix aspersa*, which were described in the previous paper of this series, and which might be thought due to a faulty technique, I have found in *Limax agrestis* especially the batonettes are very regular in size and in the amount of curvature. Faulty preparations never seemed to alter the size or curvature of the rods. The rods being stuck on an archoplasmic sphere I at first naturally thought that bad fixation of the sphere might cause the curvature of the rod to alter. *Limax agrestis* was a good form in which to study this, and my observations reinforce me in the view already taken after my "intravital" tests (previous paper, p. 597) that the rods in Helicids especially may vary in size, number, and curvature to a remarkable degree.

For variation in chondrioplasts of *Arion rufus* compare Pl. XX, fig. 32, etc., with fig. 38 in the paper of Fauré-Fremiét (3). In one case the batonettes are straight or banana-shaped, in the latter case they are bent circular.

The Fate of the Chondrioplasts in the Maturation Divisions.

In my previous paper I was unable to come to a satisfactory conclusion with regard to this question. Fauré-Frémiet (3), in his valuable paper, figures a maturation division metaphase showing the batonettes at the poles of the spindle. Now it has also been suggested that the batonettes have something to do with the formation of the amphiaster (3), and up to date no satisfactory account of this important point has been given. After carrying out some experiments, I believe I have ascertained correctly why some authors are at variance, and what becomes of the batonettes during division. If the Pulmonate ovotestis is left overnight in chromo-osmic fixatives, and stained as directed in my last paper (twelve hours iron alum, twenty hours in hæmatoxylin), the batonettes are generally not to be found during metaphase, and they disappear during the prophase.

If the material is fixed several days, as for Benda's method, in chromo-osmic fixatives, and left twenty-four hours in mordant and twenty-four hours in hæmatoxylin, the chondrioplast rods are found attached to the asters, as drawn by Murray (1) and Fauré-Frémiet (3).

It is evident, therefore, that during prophase of the maturation kinesis, the batonettes as well as the mitochondria lose much of their affinity for stains, and cannot be demonstrated without recourse to specially heavy chromatisation of the material, and long mordanting and staining.

These facts show :

(1) That the chondrioplast batonettes, unlike the mitochondria, are attracted specially by the centrosomes.

(2) That the chondrioplast batonettes undergo some chemical change during the prophase of division, and do not resume their normal reactions to fixatives and stains until after the formation of the spermatid nucleus.

(3) That the chondrioplast rods are like the mitochondria, in that they become changed somehow during the maturation

divisions, and that this all supports my contention that the chondrioplast batonettes are somewhat akin to mitochondria.

(4) That the chondrioplast rods, being demonstrable by special methods during maturation divisions, do not take direct part in the formation of the amphiaser, though it is possible that some substance in them might have been withdrawn to give rise to part of the amphiaser.

(5) That since the chondrioplast batonettes disappear altogether (with certain staining methods (1)) during division, while the mitochondria, though also affected, do not become altogether lost with these weaker staining methods, it therefore follows that the chondrioplast elements are not quite similar to the mitochondria in their behaviour during karyokinesis and in their chemical nature.

The Micromitochondria.

One object in carrying out an examination of all the Pulmonate forms that I could procure, was thoroughly to examine the remarkable micromitochondria. The latter were first described in my previous paper. The question as to their possible occurrence in other pulmonates besides the *Helicidæ* is important.

In the form *Helix aspersa* further study shows that if the cell after staining in iron hæmatoxylin is differentiated, there is a stage which can be reached at which the micromitochondria stain less heavily than the macromitochondria. In Pl. 17, fig. 19, I have drawn a spermatid of *Helix aspersa* showing the micromitochondria at *M*¹.¹ The micromitochondria are paler than the macromitochondria (*M*²); at first it may be thought that this paleness is due to the fact that the latter being larger than the former take more time to differentiate, and so appear darker. I do not think this is all; in Pl. 17 fig. 19, at *X*., is a micromitochondrium, staining palely, and at *Y*. is a macromitochondrium hardly larger than the granule at *X*., but staining quite blackly. The micromito-

¹ Good microphotographs of these have been obtained.

chondria cannot be said to stain less heavily than the macromitochondria, because they are less exposed to the stain. The micromitochondria appear to be less dense than the macromitochondria, and the attractive force exerted either by the post-nuclear plate or the anterior centrosome causes the plasmatic elements to be arranged in order of density or specific gravity. It might then be suggested that the mitochondria of *Helix aspersa* vary greatly in size in the individual cell, and that such a force tends to sort them out in order, the smaller ones being near the nucleus. In all the clearest cases I could find there was no even transition between micromitochondria and macromitochondria. The division as drawn in my Fig. 19, on Pl. 17, was very clear and sharp. Moreover, the individual size of the mitochondria in a given cell of a pulmonate is fairly regular, as drawn in all my figures. (For an exception to this, see (1) Pl. 32, fig. 24A, where the macromitochondria vary somewhat in size.) I have little doubt that the micromitochondria are present in the spermatocyte, but it is not till the sorting-out force caused by the body near the nucleus is applied that these granules become apparent.

In another section of this paper I stated the view that the mitochondria of Pulmonates do not bodily form the sheath of the sperm-tail. This undoubtedly applies to the larger mitochondria, but in later stages of spermateleosis in *Helix aspersa* it appears that the micromitochondria do become bodily applied to the axial filament, which seems to have a great attraction for them.

In *Helix nemoralis* a curious condition is found (Pl. 17, figs. 20 and 21). The part which in *H. aspersa* is occupied by the micromitochondria is here taken by about the same amount of mitochondrial matter, but the grains are the same size and stain in the same way as those lower down (Pl. 17, fig. 21). Speaking correctly, there are no micromitochondria as found in *H. aspersa*, but there are granules which act similarly to the micromitochondria. Attention should be drawn to the case of *Arion hortensis* (Pl. 17, fig. 22, *M.X.* and *M.Y.*),

where the mitochondrial granules, though of the same size throughout, are always sorted out into two nearly equal halves, which take up proximal and distal positions in the spermatid. In *H. nemoralis* the proximal mass of granules near the nucleus is never so large as the distal mass. In the case of *Testacella haliotoïdes*, the mitochondria rarely divided into separate masses, but become strewn evenly along the axial filament (Pl. 17, fig. 27).

In later stages in *H. nemoralis* the distal mass (Pl. 17, fig. 21, *M.Y.*), becomes cast down the lower length of the spermatid, while the proximal mass keeps its position. The chondrioplasts remain about half-way between for some time, but gradually pass down the tail.

I examined *Helix rufescens* (Pl. 17, fig. 23). In some cases, just behind the nucleus, one finds a greyish zone, which may represent the micromitochondria; in Pl. 17, fig. 23, *M.¹*, this collar is very plain. In many other spermatids no such zone can be found, and there is great difficulty in making quite sure whether this area behind the nucleus is really formed of granules.

Besides *Helix aspersa*, *H. nemoralis*, and *H. rufescens*, I examined *H. arbustorum* and *H. cantiana* (?). In *H. arbustorum* there were no micromitochondria, but the spheres were strewn evenly along the length of the cell. The other species, *H. cantiana*, seemed to be like *H. rufescens*, and I am uncertain as to whether micromitochondria were present or not, for their germ-cells were too small for successful study. As far as the Helicids are concerned, it will probably be found that completely differentiated micromitochondria are only present in the larger species. Owing to lack of material I have been unable to examine the case in *H. pomatia*. In other forms I found that in *Limax agrestis* the axial filament was often surrounded by a granular coat (Pl. 16, fig. 8, *M.²*). In other cases (Pl. 16, fig. 7) no coat could be found, but such cases as that drawn in the previous figure were so clear that I believe there is good evidence for supposing that micromitochondria, though

extremely minute, may be found in other forms of Pulmonates. Unfortunately, the germ-cells of the slugs are too small for satisfactory study of such a very difficult matter. It must be remembered that even in the case of *Helix*, where the germ-cells are large, other observers have overlooked the micromitochondria.

Micromitochondria in Alizarin-Crystal Violet Preparations.

The micromitochondria of *Helix aspersa* are so remarkable that many Benda alizarin-violet preparations were made in order to examine the case with this well-known stain. In this process the ovotestis of *Helix* is left for a week in Flemming with much reduced or no acetic acid at all, and then treated for twenty-four hours in a mixture of equal parts of pyroligneous acid and 1 per cent. chromic acid, then for twenty-four hours in a 2 per cent. solution of bichromate of potash. After washing for twenty-four hours sections are made in the usual way in paraffin wax and mordanted twenty-four hours in iron alum, then stained for twenty-four hours in sulfalizarinate of soda. This tints the sections a dark reddish-brown; the nucleus is in various shades of brown at the different periods, and the mitochondria are also brownish. Such stained preparations, after removal from the alizarin, are rinsed and then stained in crystal violet; they are then a dense violet colour, and differentiation is carried out in a 30 per cent. solution of acetic acid. When the violet is washed away so that the reddish shade of the alizarin can be seen appearing in the nuclei, the acetic acid is completely washed away in water. The slide is blotted, passed through absolute alcohol, where it further differentiates to bergamot oil, then to xylol balsam. Though such preparations have neither the definition nor the transparency of the iron hæmatoxylin ones made as directed in my previous paper (1), they show the nucleus and cytoplasm in colours different from the chondrioplasts and mitochondria.

Iron hæmatoxylin, of course, stains everything in black and grey.

The alizarin brown shade in the nuclei is not affected by the crystal violet, which, however, is able to stain the brown mitochondria a dense violet. That this is not a substitution of violet for brown is shown by the fact that preparations overdifferentiated in acetic acid (i. e. crystal violet washed out too much) have the mitochondria faintly brown; the violet simply smothers the brown of the mitochondria and chondrioplasts, but is easily removed from the nuclei on washing in acetic acid. On Pl. 18, fig. 34, I have drawn a spermatid as nearly as possible to the colours it stained. The macromitochondria are deep violet; the archoplasm, around which the violet chondrioplast rods are grouped, stains light reddish-brown; the nucleus is very dark reddish-brown, the posterior centrosome blue to reddish-violet, the cytoplasm is light brown, while, finally, the micromitochondria stain a purplish-brown—if anything, they tend to be more purplish-violet than brown. The reason for this is probably the same that caused the micromitochondria in Pl. 17, fig. 19, to stain grey. They are possibly less dense than the macromitochondria, so the acetic acid caused the crystal violet to be extracted more from them than from the macromitochondria. The drawing of the spermatid on Pl. 17, fig. 34, was made by enlarging the camera lucida outline to twice the original size (now $\times$ circa 8000). The other drawings on this plate are $\times$ 4000, and are to the same scale as all the other drawings of these stages. The figures 32, 33, 35 of Pl. 18 were drawn from cells in the same region as the spermatid in Fig. 34.

Changes in the Mitochondria during the Spermatocyte Divisions and during Other Stages.

If the plates of this paper be examined it will be seen that in the metaphases of mitotic division (Pl. 16, fig. 4 and Pl. 17, fig. 24) the mitochondria are of the hollow sphere variety.

Moreover, in the critique of corrosive sublimate acetic fixation given in the table on page 233, it will be seen that I have remarked that the mitochondria are most susceptible to destruction during cell division. The briefest study of the staining and fixing reactions of the mitochondria show that during division of the cell some change takes place in these bodies. In some cases the change may be also one of growth in size (e. g. Pl. 16, fig. 4). Generally it is one of stainibility. The mitochondria generally stain less heavily, and are revealed as spheres containing a chromophobe, medullary zone. In corrosive acetic fixed material, stained in iron hæmatoxylin, cells in division and about to divide either have no mitochondria visible or the latter are much distorted; other cells quite near, in growth stages, and whose mitochondria are equally exposed to the fixative, have these bodies fairly well preserved. The corrosive acetic test shows that in Pulmonates there are two periods when the mitochondria change: (1) During the maturation divisions, when they become less resistant to injurious fixatives; (2) when they have grouped around the spermatid axial filament in the stage of Pl. 18, fig. 27, etc., when they become extremely resistant to injurious fixatives.

In the Discussion (p. 247) I have attempted to throw a little light on these interesting and important periods.

The Affinities of the Helicidæ, Limacidæ, Arianidæ, and Testacellidæ judged from a Study of the Cytoplasmic Inclusions.

In a previous paper (4) on the Lepidoptera I showed that the mitochondrial elements, though slightly different in different species, did not supply sufficient reliable evidence for classifying groups of moths or butterflies. The same is even more evident with regard to the Pulmonate Mollusca. It will almost certainly be found after more critical studies have been made of numbers of species from other groups, that with probable rare exceptions, the mitochondria will

rarely provide any certain basis for settling a disputed question of specific differences between any two animals, or even for arranging a tree of ancestral relationship in various groups of animals.

The mitochondria in all the pulmonates are much alike, so much so that they provide no method in themselves of distinguishing between species. The smaller or micromitochondria when they can be demonstrated are characteristic, but since I have not had the opportunity of examining *H. pomatia* and other larger Helicids, I cannot bring forward any evidence with regard to any specificity they may have in different forms. The post-nuclear elements show that the Limacids and Helicids are alike in having these bodies slightly developed, while *Testacella* is intermediate between *Limax* and *Arion*. The chondrioplasts do not provide reliable evidence; as I have already remarked, in some forms they tend greatly to vary. Generally speaking, *Testacella* has batonnettes more like some types found in Helicids. It never has batonnettes like those of *Arion* or *Limax*. I have already said that the P.N. apparatus tends to establish relationship between *Limax* and *Helix*; the chondrioplast, on the other hand, in *Helix* is not like that of *Limax*, and this applies especially to the number of rods.

When one views the whole matter, taking into consideration chondrioplasts, mitochondria, and post-nuclear apparatus, it appears that in the Order Pulmonata one finds in every species examined the same individual cell organs, but these cell organs do not all conform to the same type, and, moreover, they appear to have no direct inter-relationship as regards the larger size of one making up for smaller size of another and vice versa, in any two cells in two separate species. It must be confessed that there is no accurate method of judging this, but that is the general impression I have gathered. For instance, why should the *Arion* spermatid in Pl. 16, fig. 16, have such a large P.N. apparatus, while the Helicid spermatid in Pl. 17, fig. 20, has such a small one? Did we know the function of this body we might endeavour to frame an

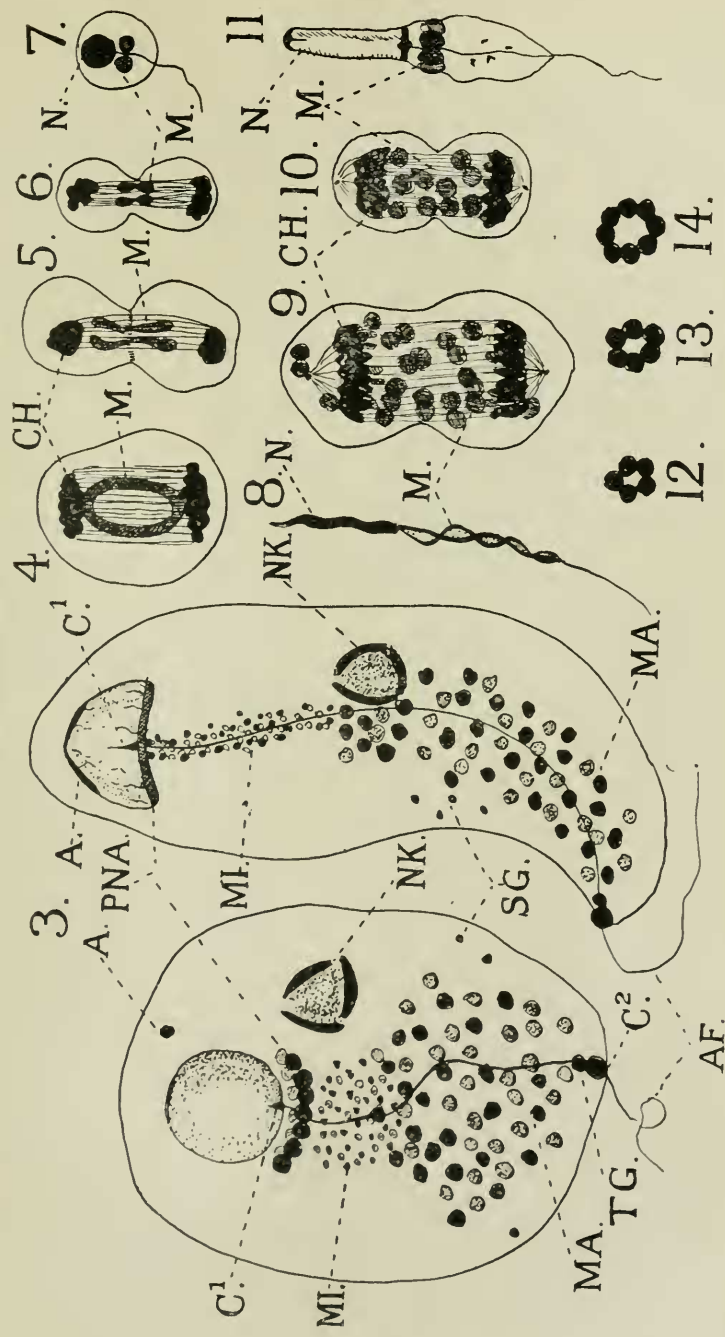
answer, but I myself am at a loss for an explanation. It would be interesting to try to follow such a cell body in all the Orders of Molluscs, if it be present. It might possibly have phylogenetically been derived from certain mitochondria, or it might represent a more important body in other Molluscs. For the present the evidence is too scanty.

Staining Affinities of the Plasmatic Bodies. (See Text-fig. 3).

With regard to the fixing and staining reactions of the various plasmatic bodies, a good deal can be learnt by experiments. The pyronin and methyl green stain introduced by Pappenheim (5), is stated by Corti and Ferrara (6) to stain chromatin green and cytoplasm red. But in Flemming and Hermann material the reverse takes place, according to the latter observers. I have made many trials with P.M.G. (pyronin and methyl green), and generally, though not always, find that after Fleming-without-acetic acid and Champy, the chromatin does stain green. Corti and Ferrara cannot have

Figs. 3-14.—Diagrammatic plan of the bodies which have been found in Pulmonate germ-cells. The two figures, one on each side of the numeral 3, are spermatids in different stages. *A.* Acrosome. *N.* Nucleus. *C.* Head centrosome. *P.N.G.* Post-nuclear granules. *N.K.* Chondrioplasts and archoplasm. *M.I.* Micromitochondria. *M.A.* Macromitochondria. *C'* Tail centrosome. *A.F.* Axial filament. *T.G.* Terminal granule (a mitochondrion probably). *S.G.* Siderophile granule. Figs. 4-8, five stages from spermatogenesis of a scorpion, *Centrurus elixicanda* (Wood), after Wilson (8). Fig. 4 shows first spermatocyte anaphase, chondriosomes (mitochondrial matter) in form of a ring (*M.*), chromosomes (*CH.*) at poles. Fig. 5 shows ring finally dividing. Fig. 6 shows second spermatocyte division telophase, final division of the chondriosome rods (*M.*). Fig. 7, young spermatid with two chondriosome bodies (macromitosome). Fig. 8 shows early stage in elongation and twisting of the macromitosome (double nebenkern of Wilson) to form the spiral envelope of the flagellum. Figs. 9-14, three stages in spermatogenesis of another scorpion, *Opisthacanthus elatus* (Gervais), after Wilson (8). Figs. 9 and 10 are first and second spermatocyte divisions, showing chondriosomes being divided between daughter-cells in the normal manner without a hoop as in *Centrurus*. Fig. 11, early spermatid showing macromitosome (nebenkern organ) at *M.* Figs. 12, 13, 14, polar views of macromitosome showing variation in number of chondriosomes (mitochondria) in individual spermatids (see p. 244 of this paper).

TEXT-FIGS. 3-14.



washed out their material properly. To get a good stain, the Flemming or Champy fixed material must be left overnight in the mixture. With regard to the reactions of this stain my friend, Dr. S. G. Scott, of the Histology Laboratory, writes to me: "Methyl green stains nuclear network, and mucin only, whether in weak or strong, or neutral or faintly acid solution. That is its value as a reagent. If there are any traces of alkali you increase its action, as also by mordanting with tannin or sulphur, or having iodine still in your section. Pyronin a basic dye, stains all that methyl green stains, and much more besides, on account of its greater dyeing power; but by using a mixture of the two dyes, with excess methyl green, the nuclei are almost pure green, while the other basophil parts of the cell are red with the pyronin. The excess methyl green keeps the pyronin out of the nuclei. Other green basic dyes will not act in the same way, because of their greater dyeing power. I have tried most of these of suitable shade, and failed to get the P.M.G. effect, as the green dye will not limit itself to the nuclei. Thus, while in an Ehrlich or other alum hæmatoxylin, toluidin blue, thionin or safranin preparation, we get all basophil things in one colour, here we find them split up into two sub-groups, the methyl green stainers, and the rest.

"For the practical cell anatomist, the advantage of the method is that it gives a certain distinction independent of shape, between a true nucleolus and a nodal point in the nuclear network, the former always red, the latter always green to black (blackish is a mixture of the two colours)."

That this is of great value I have no doubt, especially in connection with the nucleus. It is a well-known fact that from the spermatid to the formation of the ripe sperm (the spermateleosis stages) the nucleus undergoes profound staining changes. The spermatid nucleus, in pyronin and methyl green preparations is at first green, and gradually becomes bright red, and, finally, again becomes green. Van Geison's picric-acid-fuchsin stain is admirable for showing these changes, used with iron hæmatoxylin. Unfortunately, such

FIXING REACTIONS OF VARIOUS BODIES IN PULMONATE SPERMATID.

Fixative used.	Acrosome.	Nucleus.	Post-nuclear granules.	Mitochondria and chondrioplasts.	Tail granule.	Tail centrosome.
Flemming-without-acetic acid (Gatenby): Chromic acid Osmic acid Champy's fluid: Chromic acid Bichromate of potass. Osmic acid	All elements about equally well fixed. The introduction of bichromate of potassium in the Champy tends to make the preparations to stain less well, especially the nuclei and amphister.					
Corrosive sublimate acetic acid	Well preserved after adherence to nucleus	Well preserved, but not so well preserved as Champy with Champy or P.W.A.	Could not be found in spermatocyte; good after adherence to nucleus; washed-out appearance	Mitochondria ill-defined, but not always destroyed. Cytoplasm vacuolated. During cell division mitochondria generally dispersed. Better during spermatocytosis. Chondrioplasts distorted, but nettes tend to coalesce	Generally absent; when perceptible ill-defined	Good.
Carnoy's fluid: Alcohol Chloroform Acetic acid	Badly preserved, often quite destroyed	Well preserved, and stain well	Preserved in much the same way as by corr. acetic, but often destroyed	Dispersed completely	Absent	Fairly good.

delicate stains as P.M.G. and Ehrlich-Biondi do not work at all well after chromo-osmic, or chromo-bichromo-osmic material, and as many cell inclusions are only properly fixed in the latter media, one at once strikes difficulties.

In Text-fig. 3 I have given two semi-diagrammatic figures of spermatids to show the various cell bodies mentioned in my diagnosis of staining and fixing reactions. When one remembers that several late observers using good mitochondrial stains have overlooked at least two categories of cellular bodies now described by me, it will be understood what difficulty I have had in examining the staining affinities of bodies only with difficulty to be discovered in the best iron alum hæmatoxylin preparations.

In the first place with regard to fixation, Champy and Flemming's fluid without acetic acid are found to preserve perfectly all inclusions. The only objection to these fluids, from the fixation point of view is the fact that they are often too "strong" for the material. For instance, Champy undiluted rarely gives good preparations of the Testacellid ovotestis, but when diluted with distilled water by one-fourth it gives perfect results. In some ways the same applies to the Flemming without acetic acid mixture. My very best preparations of Helicids were got by leaving small pieces of the ovotestis overnight in this mixture diluted by one-sixth or more.

The commonly used corrosive sublimate acetic fixative is unreliable and worthless where delicate cytological results are a *sine quâ non*. I really only use this as a sort of control to my other chromo-osmic material. Very bright preparations can be made after this fixative, but if only a general chromosome-spindle fixation is wanted Bouin's picro-formol-acetic is superior, especially when diluted by one-third. I consider Petrankewitsch's fixative, which was really due to Gilson (the former modified the latter's formula), is superior in every way to corrosive acetic, even for whole mounts of embryos, marine animals, etc.

Of course, all the mercury bichloride acetic acid fixatives completely distort and often destroy plasmatic structures of delicate objects, probably only preserving them well in some mammalian tissues (see Waklin Barratt (7)). Even in the latter case I can only say that observers who set out to study mitochondria and other delicate plasmatic structures using ordinary fixatives are doing work which will not be of much use to serious students of the questions of the cell and heredity and general cell problems. To use a simile, such workers are only studying the "bones" of the cell after they have torn away most of the "flesh."

In my table of the effects of corrosive acetic on the cell it will be seen that in the growth stage the mitochondria are generally badly preserved, and in the prophase and during mitosis they are often quite dissolved away. After the spermatid tail is formed and when the granules become grouped around the axial filament, the mitochondria are generally good, but the whole cell has a distinct vacuolated washed-out appearance. The chondrioplast body, especially in the spermatid, is very badly preserved. The acrosome and P.N. granules after adherence to the nucleus are good, but their edge is ill defined and never so well preserved and sharp as with Champy or with F.W.A. Because of the general distortion of the cytoplasm the P.N. granules cannot be made out with any certainty in the spermatocyte.

In Carnoy material there is a general exaggeration of what occurs in corrosive sublimate acetic fixed material. Owing to the accessibility of the cells of the ovotestis to the fixative and the intense fat-dissolving powers of the mixture, the fixation of entire plasmatic bodies is extremely bad. The mitochondria and other plasmatic elements are either quite dissolved away or extremely distorted. The nuclei are well preserved, and the material generally stains well, but is, of course, absolutely useless from the cell inclusion point of view.

Moreover, there is little doubt that alcoholic acetic acid fixatives wash out a great deal of the more soluble contents of

the entire cell. One remarkable fact is that in the most perfectly fixed mitoting cells, in preparations made by chromo-osmic mixtures, the spindle-fibres are often hardly perceptible as such; instead, one finds a clear space in the cell in which one may make out faint regions where the cytoplasmic currents appeared to be flowing when the cell was fixed.

The addition of acetic acid gives a "good" spindle-fibre fixation. That is to say, the fibres of the amphiaser are marked. Do spindle-fibres exist? Or are what we suppose to be spindle-fibres altered and distorted protoplasmic currents? It is a significant fact that substances such as acetic acid and alcohol tend to accentuate the fibrous appearance of the amphiaser. At present I do not wish to add anything to the above remarks, but the matter is worthy of careful attention, and will need special experimentation.¹

Histologists are wont to use the terms "oxyphil" or "eosinophil," "amphophil," and "basophil," to denote the manner in which different parts of the cell become stained. It might then be asked, How do the mitochondria stain? Are they basophil, amphophil, or oxyphil? If the table on page 237 be consulted it will be seen that the mitochondria generally stain so vaguely with elective stains that it is impossible to say how they become coloured. With such a common stain as Ehrlich and eosin they stain a vague purplish; prolonged immersion in the basic stain gives a bluish colour to the spheres. On the other hand, prolonged staining in eosin followed by Ehrlich or toluidin blue gives a reddish tint to these spheres. It is a fact that use of the common specific stains does not satisfactorily solve the question. In a well washed-out chromo-osmic preparation it is possible to get what would generally be termed a successful Ehrlich and eosin, or Ehrlich and Biebrich scarlet stain; that is, the nuclei and karyosomes are in shades of blue, while the plasmosome and

¹ In their "Étude cinématographique de la division cellulaire," Comandon and Jolly clearly show spindle-fibres in the living dividing Triton leucocyte. 'Journ. Phys. et Path. Gén.' tome xvii, Pl. I. fig. 16.

STAINING REACTIONS OF VARIOUS BODIES IN THE SPERMATID OF PULMONATES.

Stain used.	Acrosome.	Nucleus.	Post-nuclear granules.	Mitochondria and chondrioplasts.	Tad granule.	Tad centrosome.
Pyronin and methyl green	Dark greenish-grey	Green, grey, to red, according to stage of spermatocleosis	Black in spermatocyte; dark greenish-grey in spermatid	Ill-defined greenish-grey	Not well-defined	Dark grey to reddish.
Eosin and toluidin blue	Bluish	Reddish to blue, according to stage of spermatocleosis	Blue	Reddish, ill-defined	Not defined	Bluish to purple.
Iron haematoxylin and Van Gieson	Black	Reddish, grey, yellow to black, according to stage of spermatocleosis	Black	Mitochondria greyish to black, generally grey; chondrioplasts black	Black	Black.
Ehrlich's haematoxylin and eosin	Blue	Blue to reddish, according to stage	Bluish	Purple or bluish	Not defined	Blue.
Ahzarin and crystal violet	Violet	Reddish-brown	Violet	Violet; archoplasm reddish-yellow	Violet	Reddish-blue to violet.

the ground of the cytoplasm are in pink, but the mitochondria do not show, or are very faintly pink after prolonged counter-staining. I conclude, therefore, that the colour specificity of the mitochondria probably cannot safely be worked out by using the ordinary cell stains. If crystal violet alone is used on Benda fixed material, the nuclei as well as the mitochondria are violet; but by using alizarin first the violet is generally kept out of the nuclei. It could not, therefore, be stated that the violet itself is specific for any cell element.

The acrosome invariably shows preference for basic dyes, and can undoubtedly be classed under basophil substances. The P.N. granules are the most chromophile of all the plasmatic elements, and in the spermatocyte stain as heavily and in the same way as the chromosomes. From the staining reactions of the P.N. granules I believe one cannot class the latter with the mitochondria. The chondrioplasts almost always behave like the mitochondria, except that it is always a little more chromophile in rest and growth stages, the stain remaining in it for a long time during differentiation, while the mitochondria are grey. This apparent chromophility may be due to the fact that it is denser than the mitochondria, though if the chondrioplast is denser than the latter one would not expect it to behave as it does in spermateleosis (e.g. Pl. 17, fig. 22). Though it should be borne in mind that in regard to the position the chondrioplasts take in spermateleosis, there is the archoplasm in the centre, which would probably alter the behaviour of the whole chondrioplast apparatus. I firmly believe that the chondrioplast batonnettes are chemically allied to the mitochondria.

In my previous paper (1) and in Pl. 17, fig. 20, *T.G.*, I have drawn a granule often associated with the tail centrosome. Since I described this granule in my first paper I have come to the conclusion that it is a mitochondrion; I have called it the terminal granule, and in certain spermatid bundles it is found in every cell. (See (1) Pl. 33, fig. 36.) This granule is possibly carried into the position in which it is found

simply by accident, but it is very difficult to say for certain. It acts and stains like the mitochondria.

These staining and fixing experiments have caused me to take the view that the spermatid of the Pulmonata contains several categories of plasmatic bodies whose chemical constitution, and probably density, is different in each case. A glance at Text-fig. 3 will serve to show how complicated may be the plasmatic elements of the spermatid. At the present time, despite a good deal of work, there is no certain evidence as to the part played by any of the plastid bodies. There are also granules, described by Bolls Lee, which resist acetic acid fixatives and which are siderophil—i. e. stain intensely in iron alum hæmatoxylin. Finally, it may be said under the heading of this section of the present paper, that in the chromo-osmic fixatives, properly diluted and devoid of acetic acid, we have reagents which will fix probably all the visible contents of the cell. It is to be doubted whether the spermatid of the Helicid contains bodies other than those drawn in Text-fig. 3.

DISCUSSION.

The most important facts that can be deduced from a study of the mitochondria in the Pulmonate Mollusca are as follows:

(1) No rigid synchronism exists between the stage of evolution of the nucleus in the growth period of the spermatocyte and the state of development of the mitochondria. (See the previous paper on the Pulmonata (1).)

(2) Though the entire bulk of the mitochondrial matter in a number of cells of the same generation and stage may be nearly the same, the individual granules forming this bulk may not be the same size in all the cells (1).

(3) The cell bulk and mitochondrial bulk have no rigid relationship, for one animal may have mitochondria forming a greater bulk than another whose cell at the same stage is approximately of the same size. (Compare *A. ater* and *T. haliotoides*.)

(4) The fact that in a much divided cell generation (spermatogonia (1)) the mitochondria tend to be few and large shows that in cell division the individual mitochondria are not themselves divided by any form of fission between the daughter-cells, but are distributed bodily in a haphazard manner. No certain ground for comparison in this way exists between chromosomes and mitochondria.

(5) The size of a given animal form gives no clue as to the probable size of its mitochondrial elements: e.g. among the eight pulmonates examined *L. agrestis* was nearly the smallest in body size; its mitochondria were nearly the largest of all the species.

(6) The discovery of two kinds of mitochondrial bodies in *H. aspersa* shows that whatever be the function of these bodies it is such as to call for a division of labour in certain cases.

With regard to other forms, Wilson's (8) discovery in *Centurus* (see Text-figs. 4-14) shows that—

(7) In certain rare cases there is a mechanism whereby the mitochondrial matter is equally divided among the four spermatids.¹

(8) Regaud (9) first pointed out that during the metaphase, especially of the spermatocyte divisions, the colorability of the mitochondria is altered. My own work supports Regaud. Duesberg disputes this (17).

(9) During the spermateleosis stages the mitochondria become changed again, being more resistant to injurious fixatives, and, later, more chromophile.

(10) My work on Lepidoptera (4) showed that the spermatogonial mitochondria may be more resistant to acetic acid and such injurious substances than are those of the growth stages of the spermatocyte.

(11) It seems evident that during growth period of the germ-cells the mitochondria become more numerous. They probably divide by binary fission.

¹ I have also discovered something similar in the typic spermatogenesis of *Paludina vivipara*.

(12) In some animals at least (insects, scorpions, pulmonates at certain stages, etc.) it is certain that the mitochondria consists of two parts—an inner and either non-colourable or faintly colourable core, and an outer shell of colourable or chromophile matter. The latter part of individual mitochondria often fuses to form threads or rods, which then lie in the apparently liquid chromophobe part formed by the running together of the inner zone. The inner or chromophobe substance in which the threads lie, is probably often overlooked by observers, except when the macromitosome is formed; it is then clear (see Gatenby, 'Quart. Journ. Micr. Sci.,' vol. 62, p. 426).

In this review of the plasmatic elements in the germ-cells of eight pulmonate Mollusca, I have shown that the mitochondria are, roughly speaking, throughout of the same general type and size; the establishment of the fact that no absolute regularity exists with regard to the numbers and size of the mitochondria at once shows that the part which these bodies play in the germ-cell cycle is not one which necessitates regular dimensions and number as do the chromosomes.

In the case of *Opisthacanthus elatus* (see Text-figs. 4-14), Wilson (8) found that the number of chondriosome elements contained by the spermatid varied as follows: Of two hundred cases, 73 per cent. has six mitochondrial spheres, 16 per cent. had five, and 11 per cent. had seven.

Comparing *Centrurus*, where division of the mitochondrial matter is caused by a regular mechanism, with *Opisthacanthus* Wilson says, "it is evident that chondriosome-material having the same origin, fate, and (presumably) physiological significance may be distributed to germ-cells by processes widely different even in nearly related animals."¹ This

¹ In *Paludina* I have lately found out the remarkable fact that the atypic spermatocytes have mitochondria like those of snails, while the typic have a few huge sausage-shaped mitochondria. "Cytoplasmic Inclusions of the Germ-Cells," Part V.

should be carefully borne in mind by those who would identify with the mitochondria functions needing regular and uniform behaviour in the germ cycle of animals.

The cell elements of Pulmonates which interest us most of all are the mitochondria, the chondrioplasts, and the post-nuclear granules. It appears that the post-nuclear granules may be concerned in the grouping of the mitochondria. This view is somewhat strengthened if one remembers that it is in the forms with the largest post-nuclear apparatus that the mitochondria are longest and closest grouped behind the ovoid spermatid nucleus. Unfortunately, it is not possible to ascertain whether the force which causes the mitochondrial granules to be grouped behind the nucleus is traceable to the centrosome or to the P.N. apparatus. The latter may not supply a kinetic energy of any description, it may have some function in the transmission of some hereditary factor, or it may have some part in fertilisation, at present I cannot produce any definite evidence. It is certain, with regard to the fate of this body during later stages of spermateleosis, that it dwindles and soon becomes a very small hardly noticeable disc near the head centrosome. In *Salamandra* (10) it is quite certain that the mitochondrial grains are as definitely grouped as in the Pulmonates, and without a post-nuclear apparatus. It may be possible that the P.N. apparatus in other forms has been confused with the centrosome. Bonnevie's (11) case in *Enteroxenos östergreni* is very curious, and definitely establishes that the centrosomes have an influence over the mitochondria. In later spermatids of *Enteroxenos* the indefinite grouping of the mitochondria is changed to a disposition in which the latter form a clear spiral. From this and such other evidence it is more likely that the post-nuclear granules are not exclusively for the purpose of shepherding wandering mitochondria into their proper places behind the nucleus; they may have some other and more important function which may be revealed by a study of fertilisation.

Duesberg (12), in a recent paper on the fertilisation in an Ascidian, gives a beautifully illustrated account of the mitochondria in the fertilisation and subsequent cleavage of the egg. The regions, myoplasm, ectoplasm, endoplasm, chymoplasm, and chordaneuroplasm in the egg of the Ascidian, so named by Conklin in his well-known work on these forms (13), are commented on by Duesberg as follows: "Pour moi, ces différentes substances, telles que Conklin les conçoit n'existent pas. Les différents aspects des diverses régions de l'œuf et des blastomères dépendent, non pas de l'existence de substances spéciales, mais d'une répartition spéciale des éléments figurés de l'ovoplasme. C'est ainsi que le myoplasm de Conklin n'est autre chose qu'une accumulation de mitochondries dans la région correspondante de l'œuf. . . ." In the same paragraph Duesberg also says: "La segmentation de l'œuf des Ascidiens nous montre que la division cytoplasmique est, comme le pense Rabl, un phénomène important, et très compliqué." Duesberg shows clearly that the regions of Conklin are formed by mitochondria, by yolk-grains, or by the absence of one or both of these from certain regions. It will be seen, therefore, that care should be exercised in the use of the words—"organ-forming substances." As far as we now know subsequent work may show that, on the basis of cytological examination¹ at least, the ground protoplasm of the egg is not different in different parts, but only the presence of special granules and droplets causes a pseudo-division into regions.

If this is true, our conceptions with regard to many questions of the "germ-plasm" will need drastic alteration. Further comment at present would be ill-timed; more forms must be exhaustively examined by means of the latest fixatives and stains.

With regard to the mitochondria, there are a number of schools of thought. Some observers rush headlong into the

¹ But from the subsequent developmental occurrences it must be different in different parts.

conception of the mitochondria as "les porteurs des caractères héréditaires du cytoplasme," or "die protoplasmatische Vererbungssubstanz"; others, while agreeing that the mitochondria may be of importance in heredity, are not satisfied with the evidence so far produced; there are even a few observers who either dismiss the mitochondria as "just lipoids" or absolutely deny their presence. It is a curious fact, that whenever a new cell element is discovered, one constantly finds critics describing such as the result of fixation or of staining.

It was not intended, at this juncture of my work, to comment in any way on these various schools of thought, but with regard to the view that the mitochondria represent that part of the idioplasm which is situated in the cytoplasm—a view supported by Benda (14), Meves (15), and many other prominent workers—it might be pointed out that :

(1) In the conception of units forming idioplasm one would be inclined to expect that such units would behave more regularly in cell division, being correctly distributed, as are the chromosomes. This is rarely the case with mitochondria.

(2) In the conception of units forming idioplasm one would also rightly be inclined to picture such units as more of a uniform size and number, the latter not influenced by, but properly compensated in, a varying number of cell divisions (*Helix aspersa* (1)).

I am quite willing to admit that these two conceptions of the units of the idioplasm might, perhaps, be unnecessary. For instance, the two figures in my previous paper (1) Pl. 33, figs. 37 and 40, where the number and the size of the mitochondria differs markedly, might be explained by supposing that the mitochondrion in Pl. 33, fig. 40, contained or represented a mitochondrion in Pl. 33, fig. 37, but to which a part of another mitochondrion had been added in growth. This view is, in my opinion, inadmissible, especially in the light of Wilson's discovery in *Opisthacanthus elatus* (8), where the spermatid sometimes receives five, six, or sometimes seven chondriosome spheres of the same size; compare this with the

case of *Centrurus*, where the mitochondrial matter is divided evenly.¹

Broman (15a), in a paper which I have been unable to consult myself, suggests that the haphazard division of the mitochondria is a mechanism for producing variation (12). Would Broman care to say that *Centrurus* had no capacity for variation since its chondriosome hoop is equally divided, while *Opisthacanthus*, also a scorpion, thanks to the haphazard division of its mitochondria, was endowed with special variational powers? Unless the haphazard division of the mitochondria can be explained satisfactorily with reference to functions which we must needs delegate in our minds to cell organs with more perfect distributory mechanism, I cannot conceive the use of raising innumerable ill-digested hypotheses concerning the mitochondria as carriers of hereditary factors of any kind. As organs for producing variation the mitochondria do not appear to me at present to meet the necessary requirements.

The view I at present take with regard to the relationship of mitochondria and nucleus is that the latter produces or is able to produce the former.

In a forthcoming paper I consider that I have shown plainly that this is the case. In the growth of the egg, the mitochondria undergo profound changes, and since the oocyte nucleus consists of moieties from both parents, the male parent as well as the female has had some part in the differentiation of the egg. It is certain that in many forms the tail of the sperm does not enter the egg, and the sperm mitochondria cannot be introduced in that way. Whatever function is performed by the mitochondria, it is a fact that the egg always contains an enormously greater bulk of chondriosome-material than the fully-formed sperm—and even if it be suggested that the mitochondria of the tail are like the nucleus of the sperm—much concentrated, it can be pointed

¹ Lately I have found that in *Paludina* the mitochondria in the spermatid vary in number from four to seven. See also Retzius' figures of Mollusc spermatozoa, 'Biol. Untersuch.' xiii.

out that in the spermatocyte and oocyte at the stages when these grains are of approximately the same size and staining power, those in the spermatocyte are immensely less numerous.

In both sexes there is a period of growth of the germ-cells when the number and bulk of the mitochondria become greater, and in many forms it is quite certain that the mitochondria appear only at the beginning of this growth stage, directly under the influence of the nucleus. It is evident, therefore, that there is no complete ground for comparing the nucleus and the mitochondria in the history of development. The nucleus must be passed on from generation to generation, a necessity which would not seem to and does not apply to the mitochondria.

It is evident that this view does not need one to believe that the tail of the sperm is all important in the question of the part (if any) played by the mitochondria in heredity.

I hold the view that even if it cannot be shown in all forms that the mitochondria only appear in the germ-cells at a certain part of the cycle, it is true that even if the mitochondria pass right through the germ-cell cycle, they phylogenetically were derived from the chromatin in the first place.

Minchin (16), in his excellent "Introductory Remarks on the Origin of Life," says, after giving a number of reasons for regarding the chromatin as the most important cell constituent: "For these reasons I regard the chromatin as the primitive living substance and hold the view that the earliest forms of life were very minute particles of chromatin, round which in the course of evolution achromatic substances were formed."

We are aware that not every cell in the animal body contains mitochondrial granules, and the presence of the latter in other cells with a more important function in the body is significant. One might suggest that the mitochondria arose in the evolution of the cell as bodies carrying out, in the cytoplasm, some function of a metabolic nature which hitherto had been carried out within the nucleus, and that this function

might not be in any way directly connected with heredity. We are quite certain that the hereditary function, if I may so call it, is not the only one of the nucleus; there are other functions of a more "domestic nature." Might not the mitochondria be connected with cell-metabolism in some way not necessarily directly concerned with heredity?

The study of the mitochondria has now been carried out well enough to tell us that these bodies are intimately connected in cell-metabolism and secretion in some gland-cells. We are tempted to remark, if the mitochondria are in some way directly connected with the factors of heredity either of nucleus or cytoplasm, what are they doing in gland-cells? A liver-cell or a pancreas-cell is as well supplied with mitochondria as a mammalian spermatocyte, and in gland-cells the mitochondria are often found to undergo great changes at different stages.

In the chromosomes we have a completely satisfactory mechanism for producing certain phenomena which breeding and Mendelian experiments have revealed, and nothing so far described by any of the mitochondria-heredity theorists has seriously brought the chromosome hypothesis into disrepute. I feel convinced that future work will only go to show that the mitochondria are products of the activity of the nucleus and that the so-called factors of heredity, whatever they may be, are borne by the chromosomes. It might be feasible to believe that the mitochondria are deputed by the nucleus to carry out in the cytoplasm important functions, but the fact that the mitochondria are almost certainly produced and influenced by the nucleus, must show us that the problem of the physical basis of heredity merely comes round again to the chromatin of the nucleus.

With regard to the changes undergone by the mitochondria during mitosis, Regaud (9), in his valuable paper on "*Le Structure des Tubes Seminifères et La Spérmatogénèse chez les Mammitères*," remarks: "Pendant la métaphase elles (les mitochondries) gardent cette répartition; mais leur grosseur augmente en même temps que leur chromaticité se modifie.

. . . Je pense que, pendant la mitose, les mitochondries se chargent temporairement de certaines substances, probablement voisines de la chromatine, qu'elles abandonnent ultérieurement."

Duesberg (17), reviewing Regaud's work, says: "Je crois devoir faire remarquer: 1. que dans les préparations à la méthode de Benda, les mitochondries des spermatocytes de premier ordre en division ne sont pas plus volumineuses que dans les mêmes spermatocytes au repos."

In many cases, at least, it seems to me that in pulmonates the size of the mitochondria during maturation karyokinesis becomes greater. I feel sure that there is a change in the chemical nature of these bodies at this stage, for their reactions not only to stains (Regaud), but to fixatives become altered. The figure in paper (1) of *Helix aspersa* first maturation metaphase showing rod-like mitochondria may be an artefact produced, because at this period of change the slightest fault in the mitochondria fixative induces collapse of the spheres, and a consequent rod-like or fibrillar appearance. ((1) *Helix aspersa*, Pl. 33, fig. 34.)

From my own independent studies on mitochondria, I feel sure that the latter change in some manner at these periods:

- (1) Beginning of growth stage of the spermatocyte.
- (2) Temporarily during maturation divisions.
- (3) During the later stages of spermateleosis.

The question of the change during maturation division is very significant. Why should the mitochondria change during division, and why should the change be most marked during the metaphase? Why also, should the mitochondria at this stage be more easily distorted than before? Something has either come out of or entered into the chondriosome spheres. We are undoubtedly at the beginning of a path, the following up of which may lead us to a better view of the function of these puzzling spheres. This change has been noted in Mammals (Regaud), in Pulmonates (all families I have studied), and in Lepidoptera the mitochondria during division seem to be altered in some way.

I cannot agree with Regaud in the suggestion that during mitosis "*les mitochondries se chargent temporairement de certaines substances.*" I think that they lose something during mitosis, not add anything to themselves. I do not think that the fibrillar appearance of the fixed mitochondria during the metaphase can be altogether explained by supposing that protoplasmic currents induced by dividing cell produces such an effect naturally.

Montgomery (18), in the Cape Peripatus, describes how the mitochondrial granules are completely sloughed off and never form an envelope to the tail. This is a most important matter, since Montgomery shows that the globule containing the mitochondria forms at first an investment to the nucleus and not to the hind region of the sperm, as is so often the case, and the entire mitochondrial matter is later sloughed off. Meves (19), commenting on this, says: "*Nicht nur wegen dieses Endresultates, sondern auch auf Grund der technischen Angaben, welche Montgomery macht, möchte ich bezweifeln, dass es sich bei den von ihm gesehenen Gebilden überhaupt um Mitochondrien handelt. Montgomery gibt an, dass die Mitochondrien sich an seinem Material, welches in Flemmingschem Gemisch und in Sublimat-Eisessig fixiert war. . . .*" Meves is incorrect in stating "*welches in Flemmingschem Gemisch fixiert war,*" for Montgomery plainly says in his paper that of his material "*some [was] fixed in strong Flemming's fluid diluted with an equal part of distilled water.*" This is very different from strong Flemming undiluted. As a matter of fact, strong Flemming diluted by half is a fairly good mitochondrial fixative, for the acetic acid is thereby reduced, while the osmic acid and chromic acid are still strong enough properly to preserve the chondriosome matter. Meves' criticism of Montgomery's work does not, therefore, stand.

Were Meves to find fault merely with the shape and size of Montgomery's mitochondria, I might be sufficiently impressed to suspend judgment on this point till another observer had worked on Peripatus, but I am quite aware that strong

Flemming with acetic, even undiluted, often preserves the mitochondria in certain parts of an organ, and I therefore do not consider that the fixation used by Montgomery can be brought forward as a reason for dismissing an important paper which seriously cripples Meves' views on the mitochondria.

I mentioned in the body of this paper that in all the Pulmonata I have studied at least a part of the mitochondrial grains are sloughed off. In *Peripatus*, Montgomery clearly and finally shows that all the mitochondria slough off. Duesberg (17), in the guinea-pig claims that all the grains form a part of the tail sheath, and therefore enter the egg. For my part, giving every respect to Duesberg's very excellent papers, I should like to see another observer work on the mammal without depending on Benda's methods. For instance, Duesberg (17), in his paper entitled "*Nouvelles recherches sur l'appareil mitochondrial des cellules séminales*," has studied *Blaps* (a beetle). He figures from Benda material the spermatid on Planche 3, figs. 10, 11, 12. The "corps mitochondrial" is a rounded violet body, apparently homogeneous except for a "zone périphérique extrêmement mince." I have no doubt that were preparations of *Blaps* made by the better Flemming-without-acetic, or Champy-followed-by-iron-haematoxylin method, Duesberg would find that the "nebenkern d'apparence homogène" was really a spireme.

In some unpublished researches carried out on the macro-mitosome ("Nebenkern") of *Tenebrio* I find that this body is, just as in the moths, a very finely-coiled spireme. Duesberg has committed in *Blaps* the same error made by Meves (15) in *Pygaera bucephala*. In Coleoptera the spireme is exceedingly fine, and easily mistaken for a homogeneous mass; therefore, careful staining is needed to demonstrate the coil.

Moreover, with regard to the mitochondria of Coleoptera I do not think they form rods as drawn by Duesberg.

From my unfinished observations on *Tenebrio* I believe

that the mitochondria either exist as spheres containing a chromophobe zone inside or else as elongated tubes containing internally a chromophobe substance. These tubes are formed by the fusion of granules, and when viewed, especially under monocular vision, look like apposed solid rods which are really hollow tubes (I use hollow in the sense that their centre is chromophobe, though it contains a fluid).

In a late paper on a beetle (*Passalus*) E. L. Shaffer (20) has also interpreted the coil of the macromitosome as a homogeneous mass with an outer border and the mitochondria as solid rods. In this he is in agreement with Duesberg, but I believe that more careful attention will confirm the above criticism of Duesberg's work on *Blaps*.

With regard to the question of the division of the mitochondrial matter in the maturation and other divisions, it is also important to notice that though the mitochondria of the spermatocyte be evenly distributed in quantity to the four spermatids this is not all that is required. Suppose there are six mitochondrial spheres in each spermatid cell, there is no evidence that these six bodies in each are quarters of an original six in the spermatocyte. It rather seems that the six are not individually and qualitatively divided in the spermatocyte. My study on the mitochondria, confirmed by the work of others, shows that the spermatid contains about one-quarter of the spheres found in the spermatocyte, and, moreover, that there is absolutely no special method for distributing the spheres. Nothing even analogous to what is found in the chromosomes can be seen in the vast majority of cases.

A brief return to the *Pulmonata* will serve also as a warning to the too hasty acceptance of the mitochondrial-idioplasm hypothesis. In the *Pulmonate*, besides the mitochondria, one finds two other sets of plasmatic bodies, chondrioplast rods and post-nuclear granules which are also approximately evenly divided.

We are certain that the chondrioplast batonnettes are cast

off the tail of the sperm, yet these bodies are just as evenly divided as the mitochondria, in some cases undoubtedly more so (*Limax agrestis*).

SUMMARY.

(1) Eight species of Pulmonates have been carefully studied, and certain stages of four more species examined.

(2) A new set of plasmatic bodies, the post-nuclear granules have been described.

(3) The P.N. granules differ from the mitochondria in their staining affinities, but resemble them in size.

(4) The P.N. granules ultimately form a plate at the rear of the spermatid nucleus. This plate, at first large, gradually shrinks synchronously with the shrinkage stages in the nucleus.

(5) The P.N. granules appear to become non-staining during the maturation division, but it is not known whether more intense chromatization of the material (Benda) might not bring them into evidence.

(6) The mitochondria of the Pulmonates so far studied are of the same general type.

(7) On p. 239 twelve conclusions are given with regard to the mitochondria both of Pulmonates and of other forms.

(8) The chondrioplasts of all the Pulmonates dealt with have been fully examined.

(9) The chondrioplasts of different genera and often of different species tend to differ a little in shape and size.

(10) The chondrioplasts during the prophase of the first maturation karyokinesis lose a great deal of their staining affinity.

(11) In many cases during the metaphase, anaphase, and telophase it cannot be demonstrated without specially heavy chromatization and staining. During these periods it changes in its chemical constitution.

(12) The centrosome is responsible for the sorting out of the chondrioplasts into two equal groups at the prophase of division.

(13) The batonette is not divided by fission, but goes to the daughter-cell complete.

(14) After the final maturation division is over, the chondrioplast rod resumes its lost staining affinity.

(15) The chondrioplast batonettes do not take direct part in the formation of the spindle.

(16) The micromitochondria have been thoroughly re-examined with iron hæmatoxylin and Benda's method.

(17) Their presence is confirmed by all tests that could be made.

(18) They seem to differ in staining capacity from the macromitochondria.

(19) The presence of micromitochondria in other forms is doubtful, because the cells are so small as to make proper examination impossible.

(20) Micromitochondria appear to be present in *Limax agrestis* and in *H. rufescens*.

(21) The mitochondria, during the maturation divisions, stain less heavily, and often become larger. The latter fact could not be demonstrated in every case.

(22) On p. 248 I have stated the periods during which the mitochondria appear to alter in staining affinity and resistance to certain fixatives.

(23) The affinities of the several species have been discussed on the basis of their cytoplasmic bodies.

(24) Tables of staining and fixing effects have been given.

(25) From the three papers already published on the "Cytoplasmic Inclusions" it will be seen that strong evidence is being collected against the view that the mitochondria take any part in the transmission of the "factors of heredity."

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EXPLANATION OF PLATES 16, 17, AND 18,

Illustrating Mr. J. Bronté Gatenby's paper on the “Germ-Cells of Some Other Pulmonates.”

LETTERING.

A. Acrosome. *A.F.* Axial filament. *C, C¹, C²*. Centrosome. *C.H.* Stray chromosomes. *C.R.* Central rod (mitochondrial). *G.E.* Germinal epithelium. *M.* Mitochondria. *M¹*. Micromitochondria. *M²*. Macromitochondria. *MX.* Upper mitochondria. *MY.* Lower mitochondria. *N.* Nucleus. *N.C.* Nurse-cell. *N.K.* Chondrioplast apparatus. *OOTE.* Oocyte. *P.N.G.* Post-nuclear granules. *P.N.A.* Post-nuclear apparatus. *R.* Residual mass. *S.P.* Outer spiral. *SPYTE.* Spermatoocyte. *T.G.* Terminal granule (probably mitochondrial).

In all the plates the figures have been drawn with the aid of a camera lucida, using a $\frac{1}{15}$ th semi-apochromatic Koritska oil immersion and compensating eye-pieces. Except where otherwise expressly stated all figures are multiplied circa 4000 diameters. With the exception of Pl. 18, all the figures were drawn from material preserved in either Champy's fluid (*Ch.*) or Flemming-without-acetic acid (*F.W.A.*) and stained in iron-alum hæmatoxylin.

PLATE 16.

Fig. 1.—Nearly full-grown spermatocyte of *Limax agrestis*. The archoplasm is surrounded by eight chondrioplast rods. *Ch.*

Fig. 2.—Full-grown spermatocyte of *L. maximus*. The chondrioplasts are more numerous than in the previous figure. *Ch.*

Fig. 3.—The bead of protoplasm which is finally sloughed off the ripening spermatozoon in later stages of spermateleosis (see Fig. 9 R). The mitochondria chondrioplasts and archoplasm generally stain badly, indicating change. *Ch.*

Fig. 4.—First maturation division of *L. agrestis*. Short chromatisation (see p. 222) so that chondrioplasts are not seen. Mitochondria pale, hollow, and have changed. *Ch.*

Fig. 5.—Spermatocyte of *L. maximus* in the prophases of the first maturation division. This cell is abnormally small, having been starved because of the propinquity of a large mass of later spermatids and spermatozoa. The chondrioplasts' rods are getting pale preparatory to disappearing (see p. 222), they have become disarranged, and the centrosome (*c.*) has divided. *Ch.*

Fig. 6.—Late spermatid of *L. agrestis* ($\frac{1}{15} 0 \times 12c$ eye-piece, reduced by half), showing formation of tail envelope (mitochondrial sheath) and the strewing of the mitochondria and archoplasm + chondrioplasts (*N.K.*). *F.W.A.*

Fig. 7.—Spermatid of *L. agrestis*, showing curiously compact grouping of mitochondria (*M.*) behind the post-nuclear apparatus (*P.N.G.*). *Ch.*

Fig. 8.—Later spermatid of *L. agrestis*. The axial filament, where it emerges from the mass of mitochondrial grains (*M.*), is seen to be covered by a granular coat (*M_c*), which may be the micromitochondria. *Ch.*

Fig. 9.—Part of the ovotestis of *L. agrestis* $\times 660$, to show the spermatids in three stages (1. 2. 3) with different propensity for the staining of their tail-sheaths (mitochondrial apparatus) at different stages. A group of spermatocytes (*SPYTE*) and a part of a nearly ripe egg (*OOTE.*) are also shown. *Ch.*

Fig. 10.—Young spermatid of *L. maximus*, showing the grouping of the mitochondria and the appearance of the chondrioplasts and archoplasm. The post-nuclear apparatus is also seen (*P.N.G.*).

Fig. 11.—*Arion hortensis*, spermatocyte at end of prophases of the heterotypic division and at a time when the post-nuclear granules are most conspicuous (*P.N.G.*). *F.W.A.*

Fig. 12.—*Arion ater*, full-grown spermatocyte showing post-nuclear granules (*P.N.G.*), chondrioplasts, and mitochondria. *Ch.*

Fig. 13.—Early spermatid of *A. hortensis* to show post-nuclear granules collected together into a mass near the chondrioplasts and archoplasm. *F.W.A.*

Fig. 14.—Spermatid of *A. hortensis* at a little later stage; the post-nuclear granules have become grouped to form a wall around one hemisphere of the nucleus. The acroblast is at *A.* *F.W.A.*

Fig. 15.—Spermatid of *A. ater* at a later stage; the post-nuclear granules have begun to fuse to form the post-nuclear plate. The mitochondria have become grouped behind this plate. *F.W.A.*

Fig. 16.—Later stage in *A. ater*; the post-nuclear plate has swollen to form a most conspicuous structure. The mitochondria are becoming grouped in the region of the axial filament. *F.W.A.*

Fig. 17.—Much later stage in *A. ater*; the mitochondria are grouped in the manner characteristic for *A. ater*. In some forms the axial filament appeared to be invested with a powdery coat (micromitochondria?).

PLATE 17.

Fig. 18.—*Arion hortensis*, later spermatid showing beginning of the sorting out of the mitochondria into two parts (see Fig. 22). At *X*. the archoplasm and the chondrioplasts mark the position at which the division starts. *F.W.A.*

Fig. 19.—Spermatid of *Helix aspersa*: the cell has been differentiated till the micromitochondria are pale grey, the macromitochondria still dense black. The division line betwixt micro- and macromitochondria is quite sharp. At *X*. is a micromitochondrium which is as large as the macromitochondrium at *Y*.; the latter stains densely black, the form is grey. *F.W.A.* diluted $\frac{1}{6}$.

Fig. 20.—Spermatid of *Helix nemoralis*; no micromitochondria could be found. At *P.N.A.* is the post-nuclear apparatus. The shape of the nucleus is very characteristic for this stage. The archoplasm and chondrioplasts are not yet in their usual position behind the nucleus. At *T.G.* is the terminal granule above the second centrosome. The former may be a mitochondrium. Dilute *F.W.A.*

Fig. 21.—Spermatid of *H. nemoralis* at a later stage; there is a group of mitochondria (*M.X.*) behind the post-nuclear apparatus (*P.N.A.*), then a clear space near which lie the archoplasm and chondrioplasts (*N.K.*), and behind this is the bulk of the mitochondria (*M.Y.*).

Fig. 22.—Group of spermatids of *A. hortensis* showing characteristic arrangement of mitochondria into two groups, *M.X.*, *M.Y.*, with archoplasm and chondrioplasts intermediate in position (*N.K.*). The heads of the spermatids (*N.*) are attached to a yolk- or nurse-cell (*N.C.*). At *SPYTE.* is a group of spermatocyte; *A.L.* is the outer or Ancel's layer of the ovotestis, at *G.E.* a germinal epithelial cell. $\times 1070$, *F.W.A.*

Fig. 23.—Young spermatid of *Helix rufescens*, to show at *M*¹ what may be a collar of micromitochondria. Dilute *F.W.A.*

Fig. 24.—Second spermatocyte metaphase of *Testacella halio-*
toïdes, showing mitochondria hollow spheres; the chondrioplast rods

are not to be seen as the material was not treated to show them. Champy $\frac{1}{4}$ dilute.

Fig. 25.—Head and upper part of the nearly ripe sperm of *Testacella*; At *C*¹. the first centrosome, at *P.N.A.* the small post-nuclear plate, at *S.P.* the outer spiral, left-handed coil, and at *C.R.* the central rod. Champy dilute.

Fig. 26.—Nearly ripe spermatozoon of *Testacella* to show at *N.*, nucleus, at *P.N.A.*, post-nuclear plate, at *S.P.*, spiral coil, and at *R.*, 1, 2, 3, and 4, several protoplasmic beads containing mitochondria, archoplasm, and chondrioplasts (*N.K.*). $\frac{1}{4}$ diluted, *Ch.* $\times 510$.

Fig. 27.—Advanced spermatid of *Testacella* showing the fine mitochondria fairly evenly grouped and strewn behind the post-nuclear apparatus. The large size of centrosome at *C*². is noteworthy. $\frac{1}{4}$ dilute, *Ch.*

Fig. 28.—Spermatocyte of *Testacella* in prophases of mitosis. The chondrioplasts have already become sorted out into two groups each around the centrosome; the chromosomes are appearing and the cell would shortly have divided. Later the chondrioplasts lose their staining power.

Fig. 29.—Young spermatid of *Testacella* to show post-nuclear granules and acroblast. $\frac{1}{4}$ dilute, *Ch.*

Fig. 30.—Spermatid at a time when the spiral is being formed. This process is taking place between the regions *X.-X.* and *Y.-Y.* At the letters *Z., Z.*, the inner rod drawn in Fig. 25 (*C.R.*) is being formed. $\times 830$ dilute, *Ch.*

Fig. 31.—Four regions of the tail of the sperm of *Testacella*; *C.* is the oldest, *A.* the next, while *B.* and *D.* are at the lower region of the tail of a fairly unripe spermatozoon (see text, p. 217). Not drawn to special scale.

PLATE 18.

All figures drawn from same section and cyst from a Benda Alizarin crystal violet preparation.

Fig. 34, $\times 8000$, other figures about half this magnification.

Fig. 32.—Bouquet stage, showing chromatin reddish-brown, cytoplasm light brown, chondrioplasts and mitochondria violet.

Fig. 33.—Head of ripe sperm; nucleus yellow-brown, tail-sheath (*T.S.*) violet.

Fig. 34.—Magnified up twice from camera lucida drawing; shows micromitochondria brownish-violet, macromitochondria usual violet shade (see text, p. 226).

Fig. 35.—Tail head just before sloughing off, containing mitochondria.